WAYNE STATE UNIVERSITY

Christian Kreipke, MA, PhD, FRSC
Assistant Professor, Anatomy and Cell Biology
Wayne State University
Room 9312, Scott Hall
540 East Canfield
Detroit, MI 48201
ckreipke@med.wayne.edu
313.577.1049 (o)
313.577.3125 (f)

January 26, 2010

To the Center for Scientific Review Coordinators:

This letter is written to confirm support from NINDS for this proposal to be submitted for review under the PA: PAR-08-233, The NINDS Cooperative Program in Translational Research, Single-component Research Projects (U01). This work was generated by both Dr. Armstead and myself in strict consultation with Drs. Thomas Miller, Technology Development, NINDS, and Ramona Hicks, Program Director, Repair and Plasticity. After meeting with her staff, Dr. Hicks supplied to me confirmation of approval of our project and budget which exceeds $500,000 per year on January 13, 2010 via email confirmation.

We look forward to the review process and if anything else is required of either myself or Dr. Armstead, please do not hesitate to contact me in the capacity of contact PI.

Sincerely,

Christian Kreipke, MA, PhD, FRSC

Form Approved Through 6/30/2012 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Clazosentan: A Novel Treatment of Traumatic Brain Injury

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PAR-08-233 Title: NINDS Cooperative Prog Translational Res Single-Component Res Projects (U01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Kreipke, Christian William	PhD	aa5930

3c. POSITION TITLE
Assistant Professor

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Anatomy & Cell Biology

3f. MAJOR SUBDIVISION
Medicine

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Department of Anatomy & Cell Biology
Wayne State University, School of Medicine
540 E. Canfield, Room 9312
Detroit, Michigan 48201

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 313-577-1049 FAX: 313-577-3125

E-MAIL ADDRESS:
ckreipke@med.wayne.edu

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☒ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
00002460	☒ No ☐ Yes	☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes — 5a. Animal Welfare Assurance No. A3310-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
12/01/10	11/30/14	$753,278	$1,126,964	$2,442,424	$3,770,409

9. APPLICANT ORGANIZATION
Name: Wayne State University
Address: Sponsored Programs Administration
5057 Woodward, Room 6402
Detroit, Michigan 48202

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1386028429A1
DUNS NO. 00-196-2224 Cong. District 13th

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Ms. Gail Ryan
Title: Sr. Director
Address: Wayne State University, Sponsored Prog. Admin.
5057 Woodward Ave, 13th Flr., Room 13202
Detroit, Michigan 48202
Tel: 313-577-2294 FAX: 313-577-2653
E-Mail: orspsmail@wayne.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Mr. Don Wonsowicz
Title: Grant & Contract Officer III
Address: Wayne State University, Spons Prog Admin
5057 Woodward Ave, 13th Flr., Room 13202
Detroit, Michigan 48202
Tel: 313-577-6734 FAX: 313-577-5055
E-Mail: ab3635@wayne.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)*
Donald J. Wonsowicz

DATE
1-28-10

Use only if preparing an application with Multiple PDs/PIs. See http://grants.nih.gov/grants/multi_pi/index.htm for details.

Contact Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian, W.

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
Armstead, William, M.	PhD	ARMSTEADW

3c. POSITION TITLE
Research Professor

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Anesthesia

3f. MAJOR SUBDIVISION
School of Medicine

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Dept. of Anesthesiology and Critical Care
3620 Hamilton Walk; 339 John Morgan Bldg
Philadelphia, PA 19104-6112.

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 215-573-3674 FAX: 215-349-5078

E-MAIL ADDRESS:
William.armstead@uphs.upenn.edu

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3d. MAILING ADDRESS *(Street, city, state, zip code)*

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: FAX:

E-MAIL ADDRESS:

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3c. POSITION TITLE

3d. MAILING ADDRESS *(Street, city, state, zip code)*

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: FAX:

E-MAIL ADDRESS:

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3c. POSITION TITLE

3d. MAILING ADDRESS *(Street, city, state, zip code)*

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: FAX:

E-MAIL ADDRESS:

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W

PROJECT SUMMARY (See instructions):

Traumatic brain injury (TBI) is reportedly the leading cause of death and disability among children and young adults. Among multiple sequelae, TBI results in three major pathologies: 1) cerebral edema which leads to a critical rise in intracranial pressure, 2) diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and 3) alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. Over 25 clinical trials aimed at the first two pathologies have been developed, none of which have been effective in the treatment of TBI. Therefore, novel studies leaading to new clinical trials are necessary. To date no one has initiated a clinical trial addressing the third pathology, hypoperfusion following TBI. The present proposal provides rationale for proceeding towards a clinical trial by implementing a novel therapeutic agent, Clazosentan, to improve cerebral blood flow (CBF) and, ultimately, cognitive outcome following TBI. In order to provide strong support for obtaining FDA Investigation of a New Drug (IND) status, we have designed this proposal to, first, test efficacy in a rodent model of TBI. Secondly, to more closely model the human condition, we will repeat the same efficacy measures in a porcine model of TBI. CBF will be obtained in rats and pigs using arterial spin labeling MRI (ASL), a technique used in the clinical setting, and cognitive outcome will be assessed using similar behavioral paradigms across species. The data gained from this work will be used to proceed towards a Phase II-B clinical trial to effectively use Clazosentan in an effort to improve the lives of those suffering the effects of TBI.

RELEVANCE (See instructions):

Currently no effective therapies have been designed to treat the symptoms of TBI. This project will test efficacy of a novel therapy, Clazosentan, for reducing the extent of hypoperfusion to the brain which will, in turn, improve outcome. In doing so, the expected outcome of this proposal will be to receive IND from FDA to move towards human clinical trial to improve outcome after TBI.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location		
Organizational Name: Wayne State University		
DUNS: 00-196-2224		
Street 1: 540 E. Canfield Street	Street 2: Scott Hall - Room 9320 and 9332	
City: Detroit	County: Wayne	State: Michigan
Province:	Country: USA	Zip/Postal Code: 48201-4050
Project/Performance Site Congressional Districts: MI-13		

Additional Project/Performance Site Location		
Organizational Name: Trustees of the University of Pennsylvania		
DUNS: 04-225-0712		
Street 1: 3451 Walnut Street	Street 2: P-221 Franklin Building	
City: Philadelphia	County:	State: Pennsylvania
Province:	Country: USA	Zip/Postal Code: 19104-6205
Project/Performance Site Congressional Districts: PA-002		

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Kreipke, Christian	aa5930	Wayne State Univ	Mulit-PI
Armstead, William	ARMSTEADW	Univ of Pennsylvania	Multi-PI
Dore-Duffy, Paula	aa0895	Wayne State Univ	Co-Investigator
Greenberg, Joel	GREENBERG	Univ of Pennsylvania	Co-Investigator
Kuhn, Donald	aa3071	Wayne State Univ	Co-Investigator
Marguilles, Susan	MARGULIE	Univ of Pennsylvania	Co-Investigator
Mueller, Patrick	dq4607	Wayne State Univ	Co-Investigator
Rafols, Jose	JOSERAFOLS	Wayne State Univ	Co-Investigator

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project
Goshgarian, Harry	Wayne State Univ	Conflict Resolution Negotiator
Smith, Douglas	Univ of Pennsylvania	Conflict Resolution Negotiator

Human Embryonic Stem Cells ☒ **No** ☐ **Yes**

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian, W.

The name of the program director/principal investigator must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

* Follow the page limits for these sections indicated in the application instructions, unless the Funding Opportunity Announcement specifies otherwise.

Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 04/01/09 THROUGH: 03/31/10

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Kreipke, Christian W*	Principal Investigator	3.00			155,000	38,750	9,261	48,011
Armstead, William	PD/PI-U. Pennsylvania	1.80			135,100	See	Consortium	Below
Kuhn, Donald (Yr 1 only)	Co-Invest	1.20			199,700	19,970	4,773	24,743
Dore-Duffy, Paula (Yr 1 only)	Co-Invest	1.20			188,230	18,823	4,499	23,322
Rafols, Jose (Yr 1 only)	Co-Invest	1.20			180,280	18,028	4,309	22,337
Mueller, Patrick (Yr 1 only)	Co-Invest	1.20			90,152	9,015	2,155	11,170
Schafer, Steven (Yr 1 only)	Res Asst	12.00			42,500	42,500	10,158	52,658
Fronczak, Michael (Yr 1 only)	Res Asst	12.00			42,500	42,500	10,158	52,658
Reynolds, Christian (Yr 1 only	Res Asst	12.00			42,500	42,500	10,158	52,658
*C. Kreipe (Yr 1 & 4: 3.0 cal mths; Yrs 2&3: 1.8 cal mths)								
SUBTOTALS						232,086	55,469	287,555

CONSULTANT COSTS			
Consulting Fees-Regulatory Affairs (Yr 4)		0	0
EQUIPMENT *(Itemize)*			
Two Automated Radial Arm Mazes @ $32,000 each			64,000
SUPPLIES *(Itemize by category)*			
Surgical supplies (catheters, syringes, etc)	75,000		75,000
TRAVEL	2 trips @ $2000/trip to Nat'l Mtg	4,000	4,000
PATIENT CARE COSTS	INPATIENT	0	0
	OUTPATIENT	0	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0	0
OTHER EXPENSES *(Itemize by category)*			
Animals (purchase & per diem costs)	167,700		
MRI Scans (initial $71,280 & subsequent $51,480)	122,760		
Publication costs	4,000		294,460
CONSORTIUM/CONTRACTURAL COSTS - Univ Pennsylvania	DIRECT COSTS	28,264	28,264
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD			**$ 753,278**
CONSORTIUM/CONTRACTUAL COSTS	Facilities & Administrative Costs	16,958	16,958
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →			**$ 770,237**

Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		287,555	29,671	30,561	52,463	0
CONSULTANT COSTS		0	0	0	35,000	0
EQUIPMENT		64,000	0	0	0	0
SUPPLIES		75,000	0	0	0	0
TRAVEL		4,000	6,180	6,365	4,371	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		294,460	4,120	4,244	4,371	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	28,264	845,741	616,227	49,832	0
SUBTOTAL DIRECT COSTS (Sum=Item 8a, Face Page)		753,278	885,712	657,397	146,036	0
CONSORTIUM/ CONTRACTUAL COSTS	F&A	16,958	462,445	369,736	29,899	0
TOTAL DIRECT COSTS		770,237	1,348,157	1,027,133	175,935	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$ 3,321,462**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Univ of Pennsylvania — Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 12/01/10 THROUGH 11/30/11

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Armstead, William*	Principal Investigator	1.80			133,426	20,265	5,998	26,264
Greenberg, Joel (YR 2-3)	Co-Invest	0.00			118,392	0	0	0
Margulies, Susan (YR 2-3)	Co-Invest	0.00			199,700	0	0	0
TBN (YR 2-3)	Technician	0.00			31,998	0	0	0
TBN (YR 2-3)	Technician	0.00			31,998	0	0	0
TBN (YR 2-3)	Technician	0.00			31,840	0	0	0
TBN (YR 2-3)	Technician	0.00			47,375	0	0	0
2 TBN (YR 2-3)	Undergrad Student @ 50% effort each	0.00			24,651	0	0	0
*Yr 1-1.8 Cal Mths; Yrs2-4-3.0 Cal Mths)								
	SUBTOTALS →					20,265	5,998	26,264

CONSULTANT COSTS		0 0	0
EQUIPMENT *(Itemize)* Lateral Fluid Percussion Brain Injury Device (Yr 2) Anesthesia Machine (Yr 2) Small Animal Ventilator (Yr 2)		0 0	0
SUPPLIES *(Itemize by category)* Camera Disks & Analysis (Yr 2 & 3 only) Research Supplies (Yr 2 & 3 only)			0
TRAVEL	Travel funds for PI to/from WSU & attendance @ Sci Mtg	2,000 0	2,000
PATIENT CARE COSTS	INPATIENT	0	0
	OUTPATIENT	0	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0	0
OTHER EXPENSES *(Itemize by category)* Animal costs-purchase & per diem (Yrs 2-3) Surgery costs (Yrs 2-3) w/surgical suite rental Scanning costs (80 pigs Yr 2 & 40 pigs Yr 3) Behavioral study costs (Yrs 2-3) Camera disk & miscellaneous supplies (Yr 3)			0
CONSORTIUM/CONTRACTURAL COSTS	DIRECT COSTS	0	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD			**$ 28,264**
CONSORTIUM/CONTRACTUAL COSTS	Facilities & Administrative Costs	0	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*			**$ 28,264**

Univ of Pennsylvania — Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		26,264	308,741	317,227	47,832	0
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		0	75,000	0	0	0
SUPPLIES		0	130,000	130,000	0	0
TRAVEL		2,000	6,000	6,000	2,000	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		0	326,000	163,000	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS (Sum=Item 8a, Face Page)		28,264	845,741	616,227	49,832	0
CONSORTIUM/ CONTRACTUAL COSTS	F&A	16,958	462,445	369,736	29,899	0
TOTAL DIRECT COSTS		45,222	1,308,186	985,963	79,730	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$ 2,419,101**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Budget Justification - Wayne State University

1. Christian Kreipke, Ph.D. (3.0 CM Yr 1; 1.8 CM Yrs 2-3; 3.0 CM Yr 4), an expert in both hemodynamic and behavioral changes in rats following TBI, will have overall responsibility for AIM 1, which deals with rat experimentation at Wayne State University contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of rats in this research project. Dr. Kreipke will also serve as the contact PI for this proposal.

2. Donald Kuhn, Ph.D. (1.2 CM Yr 1) is an expert in pharmacology and behavioral pharmacology. He will assist in designing experiments related to drug injection paradigms and will offer his expertise should issues arise from injection delivery to dosing regime.

3. Paula Dore-Duffy, Ph.D. (1.2 CM Yr 1) is an expert in assessing hemodynamic changes in the brain after input. She will assist in collecting the ASL-MRI data and will offer her expertise in interpreting the results.

4. Jose Rafols, Ph.D. (1.2 CM Yr 1) is an expert in assessing brain dysfunction following TBI. He will offer his expertise in conducting the weight-acceleration impact model.

5. Patrick Mueller, Ph.D. (1.2 CM Yr 1) is an expert in cardiophysiology and will assist in injection dosing and paradigm should any peripheral cardiovascular disruption ensue.

6. Steven Schafer, B.S. (12 CM Yr 1) is a research assistant in our laboratory and has extensive experience in collecting ASL-MRI data and will be instrumental in carrying out all data collection related to CBF.

7. Michael Fronczak, B.S. (12 CM Yr 1) is a research assistant in our laboratory and has extensive experience in both performing TBI surgery in rat and in collecting behavioral data.

8. Christian Reynolds, B.S. (12 CM Yr1) is a research assistant in our laboratory and has extensive experience in both performing TBI surgery in rat and in collecting behavioral data. Due to the time-intensive nature of behavioral data acquisition, he will assist Mr. Fronczak in carrying out nightly behavioral runs.

9. Equipment. While Dr. Kreipke's laboratory is currently equipped with an automated radial arm maze, 2 more radial arm mazes ($32,000 ea) will be purchased to enhance the ability to carry out large numbers of behavioral measurements as mandated by the scientific plan.

10. Supplies. **Surgery costs** ($75,000)-A modest request for maintaining surgical supplies such as scalpels, gauze, cotton, replacement hair clipper blades, etc. are included in the budget along with rental of the sterile surgical suite.

11. Travel. Funds to travel to University of Pennsylvania for direct interaction and to a scientific meeting are requested for the Multi-PIs and Co-Investigators. Two trips are requested in Yrs 1 & 4; three trips in Yrs 3 & 4.

12. Other Costs.
12a. **Animal costs** ($167,700) are included for the purchase of rats and per diem for survival surgery brain injury studies using ASL-MRI and behavior as indices of outcome.
12b. **Scanning costs**-Initially all 396 animals need to be scanned (396 x 1 hr x $180/hr)=$51,480.
12c. **Publication costs** in the amount of $4,000 per year is also being requested.

Consecutive year costs are calculated at a 3% increase.

Budget Justification - University of Pennsylvania

1. William M. Armstead, Ph.D. Multi Principal Investigator (1.8 CM Yr 1; 3.0 CM Yrs 2-4), an expert in hemodynamic changes in pigs following traumatic brain injury, will have overall responsibility for the Aims dealing with pig experimentation at the University of Pennsylvania contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of pigs in this research project.

Funding for the following has been budgeted for years 2 and 3:

2. Joel Greenberg, Ph.D. Co-Investigator (1.2 CM Yrs 2 & 3) will provide overall supervision for studies directed at the ASL MRI determination of cerebral blood flow in pigs after fluid percussion brain injury. Dr. Greenberg has had extensive experience in quantitative imaging in studies of ischemia and will ensure the quality of the data obtained. He will work closely with the technician who will transport the animal to the scanner, prepare the pig for scanning, and acquire the scanning data. The data obtained will be analyzed by Dr. Greenberg who will interact with Dr. Armstead in the interpretation of these data.

3. Susan Margulies, Ph.D. Co-Investigator (1.2 CM Yrs 2 & 3) will provide overall supervision of behavioral studies in the pig regarding the effects of fluid percussion brain injury. Dr. Margulies has had extensive experience in behavioral techniques for detecting changes associated with brain injury in the pig.

4. To Be Named Technician (12.0 CM Yrs 2 & 3) will provide technical support during surgical procedures associated with induction of fluid percussion brain injury in the pig using aseptic survival surgery technique.

5. To Be Named Technician (12.0 CM Yrs 2 & 3) will provide technical support during surgical procedures associated with the ASL MRI determination of cerebral blood flow in pigs after fluid percussion brain injury. This individual will anesthetize the pig, transport him to the scanner, prepare him for scanning, and will obtain all scanning data. This technician will work directly with Dr. Greenberg in analyzing the data acquired.

6. Two To Be Named Technicians (12.0 CM each Yrs 2 & 3) who will provide technical support in the determination of the behavioral effects of fluid percussion brain injury in the pig. These individuals will work closely with Dr. Margulies.

7. Two To Be Named Undergraduate Students (6CM Yrs 2 & 3) who'll help in the technical support in the determination of the behavioral effects of fluid percussion brain injury in the pig.

8. Equipment. A modest request ($75,000) for a dedicated lateral fluid percussion brain injury device, anesthesia machine, and small animal ventilator is requested since it would be cumbersome and difficult to move on a continual rotating basis the current devices used for acute studies between the Armstead lab and a sterile surgical suite for survival studies.

9. Supplies. Costs are included for the purchase of camera disks and research supplies.

10. Travel. Funds for travel to Wayne State University for direct interaction and travel to a scientific meeting each year is requested for the Multi-PI and the Co-Investigators. Budgeted are one trip in Yr 1 & 4; three trips in Yrs 2 & 3.

11. Other Direct Costs

11a. **Animals.** Costs are included for the purchase of pigs and per diem for survival surgery brain injury studies using ASL MRI and behavior as indices of outcome.

11b. **Surgery.** Rental of the sterile surgical suite and a modest request for maintaining surgical supplies (sutures, scalpels, gauze, cotton, replacement hair clipper blades, etc.) is included in the budget.

10c. **Scanning costs:** In year 2, 80 pigs will be studied, while in year 3, 40 pigs will be studied. Each pig will have three scanning sessions, first prior to TBI, then four hours after treatment with Clazosentan (six hours after TBI), and finally a repeat scan two days after TBI. Each scanning session will last for 60 minutes and will entail an arterial spin label (ASL) scan for measurement of regional cerebral blood flow and a T1-weighted image for structural information. Scans will be obtained using a 3 Tesla Siemens TIM Trio located in the Center for Advanced Magnetic Resonance and Specroscopy and managed by the Center for Functional Neuroimaging. The pig head can be scanned using an a volume transmit/8-channel receive knee coil. This scanner runs pseudo-ASL and provides whole-brain CBF with 4 mm isotropic resolution. The scan costs $350/hr which includes use of the preparation suite, and full use of the scanner suite. In year 2 the use of the scanner will be $84,000 (80 pigs X 3 scanning sessions X 1.0 hrs/scanning session X $350/hr), and in year 3 it will be $42,000 (40 pigs x 3 scanning sessions X 1.0 hrs/scanning session X $350/hr).

10d. **Behavioral Study costs**: In year 2, 80 pigs will be studied, while in year 3, 40 pigs will be studied. Each pig will have two behavioral sessions, the cost of each session is $50. In year 2 the cost will be $8,000 (80 pigs X 2 sessions X $50/session), and in year 3 it will be $4,000 (40 pigs x 2 sessions X $50/session). In addition, in year 2, $4,000 is needed to cover cost of camera disk analysis and 2 dedicated analysis stations. In year 3, $500 is required for camera disk analysis and miscellaneous supplies.

Year 4 Budget

In addition to salary requests for the Multi-PIs (Drs. Christian Kreipke and William Armstead), in the Wayne State Budget we are requesting $35,000 be included for consulting fees with Regulatory Affairs in Southfield, MI for application to IND.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Christian W. Kreipke	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME aa5930	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wayne State University	B.A.	1995-1999	Anthropology
Wayne State University	M.A.	1999-2000	Medical Anthropology
Wayne State University, School of Medicine	Ph.D.	2000-2004	Neuroscience
Wayne State University, School of Medicine	Postdoc	2004-2007	TBI

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has been primarily interested in how different endothelin receptor antagonists impact both CBF and behavioral outcome following TBI. This work includes published proof of concept data that supports the use of ETrA antagonists as a means to decrease the extent of hypoperfusion following TBI. Therefore, this proposal is a logical extension of previous work using BQ-123, a non clinically relevant ETrA antagonist, that has translational potential.

B. Positions and Honors

Positions and employment

01/97-05/97 Wayne State University, School of Medicine and Hutzel Hospital, Research Assistant, Bone Densitometry/Osteoporosis Project

09/97-09/99 Wayne State University, Institute for Information and Technology, Research Assistant, HIV/AIDS in Detroit Project

09/99-05/00 Wayne State University, Graduate Teaching Assistant, Department of Anthropology

05/00-09/00 Wayne State University, Adjunct Instructor, Department of Anthropology

09/00-08/04 Wayne State University, School of Medicine, Pre-Doctoral Research Assistant, National Institute of Drug Abuse T32 Training Grant

08/04-04/08 Wayne State University, School of Medicine, Research Associate, Dept. Anatomy and Cell Biology, Traumatic Brain Injury

04/08-present Wayne State University, School of Medicine, Research Scientist, Dept. Anatomy and Cell Biology

Other Experience and Professional Memberships

05/99-present	Member, Phi Beta Kappa
02/00-present	Member, Society for Applied Anthropology
02/00-present	Member, Society for Medical Anthropology
05/01-present	Member, Sigma Xi
05/01-present	Member, New York Academy of Sciences
03/01-03/02	Society for Neuroscience Brain Awareness Week Committee, Wayne State University, Chair
05/02-present	Member, Society for Neuroscience
05/02-05/04	Michigan Society for Neuroscience, Student Counselor
05/03	Michigan Society for Neuroscience Chapter Meeting coordinator
11/04-08/07	Sigma Xi, Wayne State Chapter, Executive Board Member
02/05-08/07	Wayne State Alumni Communications Committee, Committee Member
05/06-08/07	Sigma Xi, National, Associate Director, NorthCentral Region
03/07-present	Member, International Society for Cerebral Blood Flow and Metabolism
02/07-present	Chairman of the Board, Southfield Oncology Institute
08/07-present	Sigma Xi, National, Acting Director, NorthCentral Region
02/08-present	Full Member of The Royal Society

Honors

2002 Dean Thomas Asselin, M.D. Endowed Prize for Excellence in Psychiatry and Behavioral Neuroscience Research (Wayne State University School of Medicine)

2003 1st Place, Society for Neuroscience, MI Chapter, Poster Award

2006 Service Award For 2006 Sigma Xi National Conference

2007 Travel Award, Brain '07, Society for Cerebral Blood Flow and Metabolism

2007 Young Investigators Award, Endothelin 10, Endothelin

2010 Travel Award, Winter Brain

C. Peer-reviewed publications (from 32 selected works)

1. Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
2. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
3. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
4. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
5. Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.

6. Kreipke CW, Petrov T, Rafols JA. 2007. Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab, 26, S191.
7. Kreipke CW, Schafer PC, Rafols JA. 2008. Endothelin receptor A antagonism ameliorates hypoperfusion and enhances cognitive outcome following traumatic brain injury. Brain Injury 22:S43.
8. Rafols JA, Kreipke CW, Kallakuri S. 2008. Upregulation of endothelin-1 receptors in neurons and brain microvessels coincides temporally with a dysfunctional microcirculation after traumatic brain injury. Brain Injury 22:S44.
9. Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
10. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Hypoxia inducible factor-1□ signaling in aquaporin upregulation after traumatic brain injury. Neuros Lett. 453(1):68-72.
11. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Synapse Loss Regulated by Matrix Metalloproteinases in Traumatic Brain Injury Is Associated with Hypoxia-Inducible Factor-1α Expression. Brain Research 1268:125-34.
12. Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.
13. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. (in press) Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience.
14. Schafer PC, Schafer SM, Kreipke CW. (in press). Effects of light-dark cycle on motoric and cognitive activity: Implications for behavioral testing. Bio Behav Res.
15. Kreipke CW, Schafer PC, Schafer SM, Pirooz R, Angoa-Perez M, Rafols JA, Kuhn DM. (in press). Extent of hypoperfusion in a murine model of diffuse brain injury. J Neurotrauma.

D. Research Support

Ongoing Research Support

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: PI
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: PI
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-PI
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
William M. Armstead, Ph.D.	Research Professor
eRA COMMONS USER NAME ARMSTEADW	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	B.A.	05/79	Biochemistry
Tulane University, New Orleans, LA	M.S.	05/83	Pharmacology
Tulane University, New Orleans, LA	Ph.D.	05/85	Pharmacology
Tulane University, New Orleans, LA	Post-Doc	05/86	Pharmacology
University of Tennessee, Memphis, TN	Post-Doc	05/88	Physiology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. My lab is one of the few that has characterized differences in cerebral hemodynamic sequalae of TBI as a function of age using a piglet model, thought to be more similar to that of the human. Our focus has been one of describing mechanisms for brain injury that occur in the pediatric population that are distinct from that observed in the adult. One important mediator of damage investigated by us has been endothelin-1. Therefore, this proposal is a natural progession of my research interests towards developing a novel therapeutic for treatment of TBI in the pediatric and young adult population.

B. Positions and Honors

Positions and Employment

1988-1990 Instructor, Department of Physiology and Biophysics, University of Tennessee, Memphis, TN
1990-1992 Assistant Professor, Dept of Physiology and Biophysics, U of Tennessee, Memphis, TN
1992-1999 Assistant Professor, Depts of Anesthesia and Pharmacology, U of Pennsylvania; Department of Anesthesiology & Critical Care Medicine, The Children's Hospital of Philadelphia
1999-2009 Research Associate Professor, Depts of Anesthesia and Pharmacology, U of Pennsylvania
2009-present Research Professor, Depts of Anesthesiology and Critical Care, Pharmacology, U of Penn

Other Experience and Professional Memberships

1992- Member, American Physiological Society
1992- Member, American Society of Pharmacology and Experimental Therapeutics
1992- Member, Neurotrauma Society
1992- Member, Int. Society of Cerebral Blood Flow and Metabolism
1998- Editorial Board, Microcirculation
2001-2005 Chartered Member, AHA National Brain 2 Study Section
2002- Executive Board, ASPET Cardiovascular Division
2002- Member, ASPET Cardiovascular Division Student/Post Doc Best Abstract Award Committee

2005-2006	Ad Hoc Member, NIH DBD Study Section
2006-	Editorial Board, American Journal of Physiology: Heart and Circulatory Physiology
2006-2007	Member, Study Section for Catalan Agency for Health Technology Assessment, Spain
2007-	Chartered Member, Veterans Administration Neurobiology C Study Section
2008-2009	Ad Hoc Member, NIH ANIE Study Section
2008-2009	Member, AHA Region II Brain Study Section
2009-	Member, AHA Region I Brain Study Section
2009-	Awards Committee, Microcirculatory Society
2010-	Chair, Experimental Methods Section, AHA/ASA Int. Stroke Conference Planning Committee

Honors

1979	Phi Lambda Upsilon
1987	Sigma Xi
1994	Established Investigator Award of AHA
2003	Fellow, Cardiovascular Section of the American Physiological Society
2003	Fellow, Stroke Council of the AHA

C. Publications: (Selected from 163 manuscripts and 13 Reviews and Chapters)

Most relevant to the current application

1. Armstead WM. Role of endothelin-1 in pial artery vasoconstriction and altered responses to vasopressin following brain injury. J Neurosurg 85: 901-907, 1996.
2. Armstead WM. Role of endothelin-1 in age dependent cerebrovascular hypotensive responses after brain injury. Am J Physiol 277: H1884-H1894, 1999.
3. Endothelin induced cyclooxygenase dependent superoxide generation contributes to K channel function impairment after brain injury. J Neurotrauma 18: 1039-1048, 2001.
4. Armstead WM. ET-1 contributes to age dependent G protein impairment after brain injury. J Neurotrauma 20:105-110,2003.

Additional recent publications of importance to the field

1. Armstead WM, Nassar T, Akkawi S, Smith DH, Chen XH, Cines DB, and AAR Higazi. Neutralizing the neurotoxic effects of exogenous and endogenous tPA. Nature Neuroscience 9: 1150- 1155, 2006.
2. Armstead WM and MS Vavilala. Adrenomedullin reduces gender dependent loss of hypotensive cerebrovasodilation after newborn brain injury through activation of ATP dependent K channels. J Cereb Blood Flow Metab 27: 1702-1709, 2007.
3 Tontisirin N, WM Armstead, P Waitayawinyu, A Moore, Y Udomphorn, JZ Zimmerman, R Chesnut, MS Vavilala. Change in cerebral autoregulation as a function of time in children after severe traumatic brain Injury: a case series. Child's Nervous System 23: 1163-1169, 2007.
4. Freeman SS, Y Udomphorn, WM Armstead, DM Fisk, MS Vavilala. Young age as a risk factor for impaired cerebral autoregulation after moderate-severe pediatric brain injury. Anesthesiology 108: 588-595, 2008.
5. Vavilala MS, N Tontisirin, W Armstead, JZ Zimmerman, R Chesnut, AM Lam. Hemispheric differences in cerebral autoregulation in children with moderate and sever traumatic brain injury. Neurocrit Care 9: 45-54,2008.
6. Armstead WM, DB Cines, K Bdeir, I Kulikovskaya, SC Stein, A Higazi. uPA impairs cerebrovasodilation after hypoxia/ischemia through LRP and ERK MAPK. Brain Res 1231: 121-131, 2008.
7. Armstead WM, AJ Christine, AA Higazi, DB Cines. uPA impairs SNP and PGE_2 cerebrovasodilation after brain injury through activation of LRP and ERK MAPK. J Neurotrauma 25: 1375-1381, 2008.
8. Kiessling JW, DB Cines, AAR Higazi, WM Armstead. Inhibition of integrin $\alpha_V\beta_3$ prevents urokinase plasminogen activator-mediated impairment of cerebrovasodilation after cerebral hypoxia/ischemia. Am J

Physiol, 296: H862-H867, 2009.
9. Armstead WM, DB Cines, K Bdeir, Y Bdeir, SC Stein, AAR Higazi. uPA modulates the age dependent effect of brain injury on cerebral hemodynamics through LRP and ERK MAPK. J Cereb Blood Flow Metab, 29: 524-533, 2009.
10. Chaiwat O, D Sharma, Y Udomphorn, WM Armstead, MS Vavilala. Cerebral hemodynamic predictors of poor 6 month Glasgow Outcome Score in severe pediatric traumatic bran injury. J Neurotrauma 26: 657-663, 2009.
11. Armstead WM, Ganguly K, Kiessling JW, Chen XH, Smith DH, Higazi AAR, Cines DB, Bdeir K, Zaitsev S, Muzykantov VR. RBC-coupled tPA prevents impairment of cerebral vasodilatory responses and tissue injury in pediatric cerebral hypoxia/ischemia through inhibition of ERK MAPK. J Cereb Blood Flow Metab 29: 1463-1474, 2009.

D. Research Support

Ongoing Research Support

NS 53410 (Armstead) 6/1/06-5/31/11 4.8 CM
NIH/NINDS
Plasminogen activators and cerebral ischemic injury
The major goals of this project are to: 1. Characterize the relationship between the plasminogen activators and cerebral hemodynamics after hypoxia/ischemia, 2. Investigate the role of MAPK as the mechanism by which plasminogen activators control cerebral hemodynamics post insult; Changes in the MAPK isoform expression profile result in impaired cerebral hemodynamics and neuron cell loss, and 3. Deteremine the association between impaired cerebral hemodynamics and histopathology post insult.

HD57355 (Armstead) 3/20/08-2/28/13 4.8 CM
NIH/NICHD
Plasminogen activators and NMDA after brain injury
The major goals of the project are to: 1. Characterize the relationship between plasminogen activators and NMDA receptor activation in cerebral hemodynamics following brain injury as a function of age, 2. Investigate the role of MAPK isoforms and LRP as the mechanism by which plasminogen activators and NMDA receptor activation control cerebral hemodynamics following brain injury as a function of age, and 3. Determine the association between plasminogen activators and NMDA receptor induced impairment of cerebral hemodynamics and histopathology following brain injury as a function of age.

HL 77760 (Higazi) 4/1/06-3/30/11 0.6 CM
NIH/NHLB
tPA in traumatic brain injury
The major goals of this project are to: 1) Study the role of tPA in traumatic brain injury; 2) Identify the receptors that mediates its signal transduction effect and to develop approaches to inhibit such pathways.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Paula Dore-Duffy	Professor of Neurology
eRA COMMONS USER NAME (credential, e.g., agency login) aa0895	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Simmons College, Boston, MA	B.S.	1972	Biology
Baylor College of Medicine, Houston, TX	--	1973	Virology
Louisiana State University, School of Medicine, New Orleans, LA	Ph.D	1978	Microbiology / Immunology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. Dr. Kreipke and I have worked closely on several projects regarding blood flow, angiogenesis, and BBB. Further, I have served on numerous study sections for the National Institutes of Health, Department of Defense, and the Veterans' Administration Hospitals in the MS, immunology, and vascular biology fields. Over the years, I have had considerable experience running a research program at the R01 level and Center grant. I was recently awarded one of the MS Society's Collaborative Research Center Awards that coordinates four projects. I therefore have the background and expertise to lead the proposed research program.

B. Positions and Honors

Positions and Employment

1979-1982 Assistant Professor, Neurology and Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Neurology, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Director, UCHC MS Center, University of Connecticut School of Medicine, Farmington, CT
1977 Tenure, University of Connecticut School of Medicine, Farmington, CT
1984-1988 Co-Director, Neuroscience Graduate Program, University of Connecticut School of Medicine, Farmington, CT
1988-Pres Co-Director, Multiple Sclerosis Center, Wayne State University School of Medicine Detroit, MI
1989-Pres Chief, Division of Neuroimmunology, Department of Neurology, Wayne State University School of Medicine Detroit, MI
1988-Pres Professor, Neurology, Wayne State University School of Medicine Detroit, MI

1988-Pres Associate Professor, Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI
1996-1998 Co-Director, Detroit Neurotrauma Center, Wayne State University School of Medicine, Detroit, MI

Other Experience and Professional Memberships
National Institutes of Health, Study Section Reviewer, BINP, 2005-2007
American Heart Foundation, Reviewer, 2007
Department of Defense Neurobiology Committee A, Member, 2007
Program Committee, Winter Conference on Brain Research 2007
American Neurological Association
American Academy of Neurology
The American Association of Immunologists (AAI)
Society for Neuroscience
American Society of Neurochemistry
The Royal Society of Medicine
National Neurotrauma Society
International Society for Blood Flow & Metabolism
International Brain Barrier Society

Honors / Awards
2008 Academy of Scholars, Wayne State University
2007 Faculty Excellence in Research Award, Wayne State University School of Medicine
1998 Deputy Editor, Journal of Neurological Sciences
1998 NIH Study Section, NB4
1996 Gershenson Distinguished Faculty Fellow
1994-Pres Editorial Boards: Neurology, Microvascular Research, Journal of Experimental Microbiology and Immunology, Journal of Clinical Pharmacology
1988-1992 National Institute of Health Study Section (NSPA)
1985-1986 Visiting summer scientist Mount Desert Island Biological Labs, Salisbury Cove, Maine
1985 Kroc Foundation Endowment $50,000 to establish a yearly MS Symposium
1980 Multiple Sclerosis Society Bursar, 4th International Congress of Immunology in Paris
1979 Outstanding Young Women Scientist of America
1977-1978 National Multiple Sclerosis Society Fellowship Award

C. Selected Peer-reviewed Publications
Most relevant to the current application

Dore-Duffy, P., Donovan. C. and Todd, R.F. III. Monocyte Activation Associated Antigen MO3e in MS. Neurology. 42:1609-1615, 1992.

Washington, R., Burton J., Todd, R.F., III, Dragovic, L., and Dore-Duffy, P. Expression of immunologically Relevant Endothelial Cell Activation Antigens of Isolated CNS Microvessels from Patients with MS. Evidence for Focal Activation of Vascular Endothelium. Ann. Neurol. 35:89-97, 1994.

Washington, R. and Dore-Duffy, P. Role of Cytoskeletal Elements in Expression of Monocyte Urokinase Plasminogen Activator Receptor, Activation-Associated Antigen Mo3. Clin. Diag. Lab. Immunol. 1(6): 714-721, 1994.

Dore-Duffy, P., Balabanov, R., Washington, R., and Swanborg, R.H. Transforming Growth Factor Beta-1 Inhibits CNS Endothelial Cell Activation. Mol. Chem. Neuropath. 3:161-175, 1994

Dore-Duffy, P., Washington, R., and Balabanov, R. Cytokine-mediated activation of cultured CNS microvessel: A system for examining antigenic modulation of CNS EC and evidence for long-term expression of adhesion protein E-selectin. J. Cereb. Blood Flow Metab. 14:837-884, 1994.

Dore-Duffy, P., Balabanov, R. and Washington, R. Recovery from acute experimental autoimmune Encephalomyelitis (EAE) characterized by endothelial cell unresponsiveness cytokines and

pericytes activation. Biology and Physiology of the Blood-Brain Barrier. 57:347-351, 1996.
Dore-Duffy, P., Balabanov, R., Beaumont, T., Hritz, M.A. Harik, S.I. and LaManna J.C. Endothelial activation Following Prolonged Hypobaric Hypoxia. Microvas. Res. 57:75-85, 1999.
Balabanov, R., Dore-Duffy, P., The CNS microvascular pericyte response to hypoxia. Neurotrauma 19:1331, 2002.
Dore-Duffy, P, Balabanov R, Wang X, Beaumont T. Pericyte release of cyclopentenone prostaglandins in response to hypoxia. Microvascular Research 35; 215-226, 2005.
Dore-Duffy, P., Katychev, A., Wang, X, and Van Buren, E. CNS microvascular pericytes exhibit multipotential stem cell activity. J Cereb Blood Flow Metab. 26: 613-624, 2006.
Dore-Duffy, P. and LaManna, JC. Physiological Angiodynamics in the brain and Redox signaling. Antioxidants and Redox Signaling 9: 1363-1371, 2007.
Dore-Duffy, P., Wang, X., Mehedi, A., Kreipke, W., and Rafols, J. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurological Res. 29: 395-403, 2007.
Milner, R., Hung, S., Erokwu, B., Dore-Duffy, P., LaManna, J., and del Zoppo, G. Increased expression of fibronectin and the $\alpha 5\beta 1$ integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. Mol Cell Neurosci. 38: 43-52, 2008.
Dore-Duffy, P. Pericytes: Pluripotent cells of the blood brain barrier. Curr Pharm Des. 14: 1581-1593, 2008.
Dore-Duffy, P., Cleary, K. "Morphology and Properties of the Neurovascular Unit: The Pericyte", *in* The Blood-Barrier and Other Neural Barriers: Biology and Research Protocols, Ed: S. Nag, Humana Press Inc, Totowa (in press).

D. Research Support

Collaborative Multiple Sclerosis Research Center Award
Principal Investigator: Paula Dore-Duffy, Ph.D.
Agency: National Multiple Sclerosis Society
Period: April 1, 2007 to March 31, 2012

Poly-trauma following brain injury: towards a combinatorial therapy
Principal Investigator: Christian Kreipke, PhD
Agency: Veteran's Administration (VARR&D 1I01RX000224-01)
Period: November 1, 2009 to October 31, 2012
(Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Greenberg, Joel H.	Research Professor Neurology
eRA COMMONS USER NAME (credential, e.g., agency login) GREENBERG	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Manitoba, Winnipeg, MB. Canada	B.S.	1967	Engineering Physics
University of Pennsylvania, Philadelphia, PA	Ph.D.	1974	Biomedical Engineering

A. Personal Statement

The goal of the proposed research is to investigate the translational potential of Clazosentan, an endothelin-1A antagonist, in ameliorating hypotension and in improving behavioral outcome following traumatic brain injury (TBI). Specifically, we will use TBI models in rats (preliminary dosing and probing studies) and in pigs (a species for which a model more relevant to human TBI exists) to evaluate Clazosentan for treating TBI. I have been working in the field of cerebrovascular physiology and cerebral ischemia for over three decades utilizing ischemia models in rats, cats, and non-human primates. I have used autoradiographic, tomographic (PET, SPECT), and optical techniques to measure cerebral blood flow in models of ischemia, hypoxia, hypotension and functional activation. Early in my career I worked with Drs. Reivich and Kuhl at the University of Pennsylvania, and Dr. Wolf at Brookhaven National Laboratories to develop the 18-F-fluorodeoxyglucose technique for the measurement of cerebral glucose utilization and have used this technique to study changes in glucose metabolism in a variety of disease states and following functional activation. This has provided me with a strong background in tomographic measurements of blood flow and metabolism. More recently I have been working with Dr. John Detre from the Departments of Neurology and Radiology to migrate and validate the magnetic resonance imaging techniques for blood flow measurements routinely used in humans to the pig and the rat. Dr. Detre, who developed the arterial spin label (ASL) technique for blood flow measurement using MRI that will be used in the proposed studies, and I have been close collaborators for over ten years. I bring to this project extensive experience in the measurement of cerebral blood flow in models of cerebral ischemia and will be responsible for the ASL-MRI measurements following fluid percussion TBI in the pig.

B. Positions and Honors

Positions and Employment

1968-1968	Research Associate, Physiological Flow Studies Unit, Imperial College of Science and Technology, London, England.
1973-1975	Research Associate, Cerebrovascular Research Center, Department of Neurology, University of Pennsylvania, School of Medicine.
1975-1980	Research Assistant Professor, Dept. of Neurology, University of Pennsylvania, School of Medicine.
1980-1997	Research Associate Professor, Dept. of Neurology, University of Pennsylvania, School of Medicine
1997-present	Research Professor, Department of Neurology, University of Pennsylvania, School of Medicine

Other Experience and Professional Memberships

1978-present	Fellow-Stroke Council, American Heart Association
1997-present	Director, International Society of Cerebral Blood Flow and Metabolism

2001-2003 Chair Program Committee, Brain'03 (International Symposium of Cerebral Blood Flow, Metabolism and Function)
2005-2007 President-elect, International Society of Cerebral Blood Flow and Metabolism
2007-2009 President, International Society of Cerebral Blood Flow and Metabolism

Honors

1967-1969 Ford Foundation Fellowship
1969-1973 Medical Research Council of Canada Studentship

C. Selected Peer-reviewed Publications (Selected from 175 peer-reviewed publications)

Most relevant to the current application

Greenberg, J.H., Reivich, M., Alavi, A., Hand, P., Rosenquist, A, Rintelmann W., Tusa, R., Stein, A., Christman, D., Fowler, J., MacGregor, B., and Wolf, A.: Metabolic mapping of functional activity in man with the ^{18}F-fluorodeoxyglucose technique. Science 212:678-680, 1981.

Komatsumoto, S., Nioka, S., Greenberg, J.H., Yoshizaki, K., Subramanian, V.H., Chance, B., Reivich, M.: Cerebral energy metabolism measured in vivo by 31P-NMR in middle cerebral artery occlusion in the cat - relationship to severity of stroke. J. Cereb. Blood Flow Metab. 7:557-562, 1987.

Dezsi, L., Greenberg, J.H., Hamar, J., Sladky, J., Karp, A., Reivich, M.: Acute improvement in histological outcome by MK-801 following focal cerebral ischemia and reperfusion in the cat independent of blood flow changes. J Cereb Blood Flow Metabol 12:390-399, 1992.

Lu, D., Joseph, P.M., Greenberg, J.H., Lin, R., Mukherji, B., Sloviter, H.A.: Use of 19F magnetic resonance imaging to measure local cerebral blood volume. MRM 29:179-187, 1993.

Greenberg JH, Araki N, Karp A: Correlation between ^{99m}Tc-bicisate and regional CBF measured with iodo-[^{14}C] antipyrine in a primate focal ischemia model. J Cereb Blood Flow Metabol 14(Suppl 1):536-543, 1994.

Takahashi, K., Pieper, A.A., Croul, S.E., Zhang, J., Snyder, S.H., Greenberg, J.H.: Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. Brain Res. 829:46-54, 1999.

Shimazu, T, Inoue I, Araki, N, Asano, Y, Sawada, M, Furuya, D, Nagoya, H, Greenberg, JH.: A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia. Stroke 36:353-359, 2005

Additional recent publications of importance to the field (in chronological order)

Otori, T., Greenberg, J.H., Welsh, F.A.: Cortical spreading depression causes a long-lasting decrease in cerebral blood flow and induces tolerance to permanent focal ischemia in rat brain. J Cereb Blood Flow Metabol 23:43-50, 2003.

Buerk, D.G., Ances, B.M., Greenberg, J.H., Detre, J.A.: Temporal dynamics of brain tissue nitric oxide during functional forepaw stimulation in rats. NeuroImage 18:1-9, 2003

Durduran, T., Burnett, M.G., Yu, G., Zhou, C., Furuya, D., Yodh, A.G., Detre, J.A., Greenberg, J.H.: Spatiotemporal quantification of cerebral blood flow during function activation in rat somatosensory cortex using laser-speckle flowmetry. J Cereb Blood Flow Metab 24:518-525, 2004

Burnett, M.G., Detre, J.A., Greenberg, J.H.: Activation-flow coupling during graded cerebral ischemia. Brain Res 1047(1):112-8, 2005

Burnett, M.G., Shimazu, T., Szabados, T., Muramatsu, H., Detre, J.A., Greenberg, J.H.: Electrical forepaw stimulation during reversible forebrain ischemia decreases infarct volume. Stroke 37:1327-1331, 2006

Luckl, J., Keating, J., Greenberg, J.H.: Alpha-chloralose is a suitable anesthetic for chronic focal cerebral ischemia studies in the rat: A comparative study. Brain Res 1191:157-167, 2008

Zhou, C., Shimazu, T., Durduran, T., Luckl, J., Kimberg, D.Y., Yu, G., Chen, X-H., Detre, J.A., Yodh, G., Greenberg, J.H.: Acute functional recovery of cerebral blood flow following forebrain ischemia in the rat. J Cereb Blood Flow Metabol 28:1275-1284, 2008

Luckl, J., Zhou, C., Durduran, T., Yodh, A.G., Greenberg, J.H. : Characterization of periinfarct flow transients with laser speckle and Doppler after middle cerebral artery occlusion in the rat. J Neurosci Res 87:1219-1229, 2009

D. Research Support:

Ongoing Research Support

1-RO1 NS057400 Greenberg (PI) 03/01/08 – 02/28/11
NIH/NINDS
Functional activation during cerebral ischemia

The goal of this project is to examine the neuroprotective properties of functional stimulation during cerebral ischemia.
Role: PI

1-RO1 NS060653 (Yodh) 09/01/08 – 08/31/12
NIH
Diffuse Optics for Acute Stroke Management

The major goal of this project is to develop optical tools to monitor acute stroke patients and to demonstrate the clinical utility of these tools.
Role: Subcontract PI

ITMAT (Greenberg/Dmochowski/) 10/01/08 – 09/30/10
Institute for Translational Medicine (PENN)
Validation of Target Xenon SPECT Agents for *In Vivo* Molecular Imaging

The goal of this project is to develop protocols for attaching peptide and small-molecule targeting agents to cryptophanes so as to produce compounds that can target cancer cells or be used to monitor variety of physiological functions and thus be used in emission tomography.
Role: Co-PI

Completed Research Support

URF Greenberg (PI) 07/01/04 – 6/31/05
University of Pennsylvania Research Foundation
A Portable Device to Monitor Cerebral Blood Flow, Oxygen Saturation and Oxygen Metabolism in Patients

This grant is dedicated to building a device for the non-invasive measurement of cerebral blood flow and cerebral oxygen concentrations in head-injured patients. This device will be tested in a model of focal ischemia in the cat.
Role: PI

1-R01-HD44769-01 Hoffman (PI) 09/01/03 – 06/30/06
NIH/NICHD

Enhancing recovery of sensation after cerebral ischemia
The focus of this research project is to examine factors that optimize functional recovery and maximize the anatomical and physiological reorganization of the central nervous system following central lesions.
Role: Subcontract PI

2-RO1-NS33785 Greenberg (PI) 09/01/02 – 06/30/07
NIH/NINDS
Ischemia Induced Plasticity – Implications for Therapy

The major goal of this project is to examine metabolic and behavioral reorganization following focal cerebral ischemia of the somatosensory cortex in the rat, and develop techniques for accelerating this reorganization.
Role: PI

R01 HL077699-01 Yodh (PI) 09/01/04 – 08/31/08
NIH/NHLBI
Diffuse Light Imaging of Flow, Oxygen & Brain Metabolism

The goal of this project is to design and build a versatile multi-modality all-optical imaging probe for measurement of total hemoglobin concentration, blood oxygenation, blood flow, the cerebral metabolic rate for oxygen and changes of these parameters in the brain.
Role: Subcontract PI

CNC Greenberg (PI) 02/01/07 – 01/31/08
Comprehensive Neuroscience Center
Optical monitoring of acute stroke patients

The goal of this project is to evaluate the ability of an optical imaging device to monitor cerebral auto-regulation in acute stroke patients so that their post-stroke care can be individualized
Role: PI

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Donald M. Kuhn	Professor
eRA COMMONS USER NAME	
aa3071	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Presbyterian College	BS	1972	Biopsychology
University of South Carolina	PhD	1976	Behavioral Pharmacology
Princeton University	Postdoc	1976-1977	Electrophysiology
National Institutes of Health	Postdoc	1977-1983	Biochemical Pharmacology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has a long history of performing pharmacological studies and therefore I will aide Dr. Kreipke with his design of dosing and dosing regime for Clazosentan. Furthermore, this work will compliment our co-funded VA grant which combines our expertise to develop future therapeutics for TBI victims.

B. Positions and Honors

Positions and Employment

1983-1986-Chief, Section on Biochemical Pharmacology, National Heart Lung & Blood Institute, NIH
1985-1986-Alexander von Humboldt Fellow, Department of Neurochemistry, Goethe University, Frankfurt, Germany
1987-present- Professor, Department of Psychiatry and Behavioral Neurosciences, Center for Molecular Medicine and Genetics, and Institute for Chemical Toxicology, Wayne State University School of Medicine
1993-1994-Visiting Professor, Dept. Molecular Genetics and HHMI, Univ. Texas Southwestern Medical Center, Dallas, Texas (Sabbatical leave in Dr. T. Sudhof's lab)
1998-present- Research Career Scientist, John D. Dingell VA Medical Center, Detroit, MI

Other Experience and Professional Memberships

1994-1998 Member, NIDA-C (now NMB) Scientific Review Subcommittee
1998-2002 Member, MDCN-4 Scientific Review Subcommittee
1999- Member, Editorial Board Journal of Neurochemistry
1999- Ad hoc reviewer for MDCN-3, IFCN-7, Neurological Sciences & Disorders B, NIDA Cebra Program, and numerous SEPs for NIDA, NINDS, and NIMH
2001- National Scientific Advisory Council, American Federation for Aging Research
2004- Member, Neurobiology A Merit Review Subcommittee, Dept. Veterans Affairs
2006- Member, NMB Scientific Review Subcommittee

Honors

1985- Fellow, Alexander von Humboldt Foundation

C. Selected peer-reviewed publications (in chronological order)

(Publications selected more than 135 peer-reviewed publications and book chapters)

Wolf, W.A. and **Kuhn, D.M.** Molecular pharmacology of the neuronal serotonin transporter: Role of essential sulfhydryl groups in ligand binding and transport. J. Biol. Chem. 267, 20820-20825, 1992.

Kuhn, D.M. and Geddes, T.J. Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation: Coincident nitration of enzyme tyrosyl residues has minimal impact on catalytic activity. J. Biol. Chem. 274, 29726-29732, 1999.

Anastasiadis, P.Z., Jiang, H., Bezin, L., **Kuhn, D.M.**, and Levine, R.A. Tetrahydrobiopterin enhances apoptotic cell death following withdrawal of trophic support. J. Biol. Chem. 276, 9050-9058, 2001.

Kuhn, D.M. Dopamine and Its Modulation of Drug-Induced Neuronal Damage, E. Massaro (Ed.). In: Handbook of Neurotoxicology, Volume 2, Drugs of Abuse, Humana Press, pp 175-197, 2002.

Kuhn, D.M., Sadidi, M., Lu, X., Kreipke, C., Geddes, T., Borges, C., and Watson, J.T. Peroxynitrite-induced nitration of tyrosine hydroxylase: Identification of tyrosines 423, 428, and 432 as sites of modification by MALDI-TOF mass spectrometry and tyrosine-scanning mutagenesis. J. Biol. Chem., 277, 14336-14342, 2002.

Kuhn, D.M. and Geddes, T.J. Reduced nicotinamide nucleotides prevent nitration of tyrosine hydroxylase by peroxynitrite. Brain Research, 933, 85-89, 2002.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Verbeem, D., Liu, X., and **Kuhn, D.M.** Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci. Lett., 367, 349-354, 2004.

Thomas, D.M. and **Kuhn, D.M.** Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. J. Neurochem., 92, 790-797, 2005.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. FASEB J (FJ Express Summary), 20, 515-517, 2006.

Kuhn, D.M., Sakowski, S.A., Geddes, T.J., Wilkerson, C., and Haycock, J.W. Phosphorylation and activation of tryptophan hydroxylase 2: Identification of serine-19 as the substrate site for calcium-dependent protein kinase II. J. Neurochem., 103, 1567-1573, 2007.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. J. Neurochem., 605-616, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., in press, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., 1139, 118-126, 2008.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Increases in cytoplasmic dopamine compromise the normal resistance of the nucleus accumbens to methamphetamine neurotoxicity. J. Neurochem., 109, 1745-1755, 2009.

Kreipke, C.W., Schafer, P.C., Schafer, S.M., Pirooz, R., Angoa-Perez, M., Rafols, J.A. and **Kuhn, D.M.** Validation of a mouse acceleration-impact model of traumatic brain injury. J. Neurotrauma, in press, 2009.

D. Research Support

Ongoing (Active) Research Support

NIH/NIDA 5 R01 DA10756 04/10/07-04/09/12

Neurotoxic Amphetamines, Radicals, and 5HT Neurons

The major goal of the study is to determine the mechanisms by which neurotoxic amphetamine-derived reactive oxygen and nitrogen species alter function of dopamine and serotonin neurons through their effects on important phenotypic marker proteins in these neuronal elements.

Role: PI

NIH/NIDA 1 RO1 DA017327 04/01/05 – 03/30/10
Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines.
Role: PI

Department of Veterans Affairs Merit Award 03/15/07-03/14/11
Brain Injury by Blast Overpressure: Role of Microglial Activation
The goal of this project is to characterize microglial involvement in brain damage caused by blast overpressure. We have developed a model of blast overpressure, a form of traumatic brain injury, that allows testing of cultured cells and brain slices.
Role: PI

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).
Role: Co-I

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).
Role: Co-I

Projects completed in the past 3 years

NIH/NIDA 1 K05 DA14692 10/05/02-12/04/07
Molecular Biology of Drug Abuse
This is a senior scientist career development award.
Role: PI

NIH/NIDA 1 T32 DA07310 07/01/00-06/30/06
Neuroscience Training in Drug Abuse
This is a training grant that supports two predoctoral and two postdoctoral fellows. This training program is in hiatus temporarily. Our department experienced some significant changes in faculty re-assignment to other academic units, and several other key investigators on the T32 have left Wayne State. Therefore, we are re-configuring this training program as the Translational Neuroscience Program to reflect more accurately the current mentoring and research expertise of our departmental faculty.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Susan Sheps Margulies, Ph.D.	Professor in Bioengineering
eRA COMMONS USER NAME (credential, e.g., agency login) margulie	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Princeton University, Princeton, NJ	BSE	1982	Mech. & Aerosp. Eng'g
University of PA, Philadelphia, PA	MSE	1983	Bioengineering
University of PA, Philadelphia, PA	PhD	1987	Bioengineering
Mayo Graduate School of Medicine	Res. Fellow	1987-89	Thoracic Diseases

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

Dr. Margulies, a Professor in Bioengineering, is an investigator on the project, and will oversee all aspects of the pig behavioral assessments in proposed research plan. Dr. Margulies has over 25 years of experience in the area of traumatic brain injury research; many recent publications focus on the biomechanics of traumatic brain injury in adults and children, and assessments of cognition, memory and behavior in piglets.

B. Positions and Honors

Positions and Employment

1989-1990	Senior Res Fellow, , Mayo Med. School, Mayo Clinic, Rochester, MN
1989-1990	Instructor, Dept. of Physiology & Biophysics, Mayo Medical School, Mayo Clinic, Rochester,MN
1990-1992	Research Associate, Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN
1990-1993	Assistant Professor, Dept. of Physiology & Biophysics, Mayo Medical School, Rochester, MN
1992-1993	Associate Consultant, Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN
1993-1998	Assistant Professor, Department of Bioengineering, University of PA, Philadelphia, PA
1998-2004	Associate Professor, Department of Bioengineering, University of PA, Philadelphia, PA
2004-present	Professor Dept. of Neurosurgery, Univ. of PA, Philadelphia, PA
2004-present	Professor, Department of Bioengineering, University of PA, Philadelphia, PA
2007-present	Graduate Group Chair, Bioengineering University of PA, Philadelphia, PA

Federal Advisory Committees:

1997-2002	Member, NSF Review Panel - Bioengineering Grants, CAREER Awards, Graduate Fellowships
2002	Member, NSF Committee of Visitors -Review of Bioengineering (BES) Division
2000-2003	Member, CDC Injury Research Grant Review Committee
2001	Member, NIH Study Section ZRG1 SSS-3 (03)
2002, 2008	Member NIH NHLBI PPG Study Section
2003-4	Member (ad hoc), NIH RESP and RIBT Study Sections
2008-pres	Member (standing), NIH RIBT Study Section
2007- pres	Member, New Jersey Commission on Brain Injury Research Scientific Review Committee

Honors:
1982 Summa Cum Laude, Princeton University; 1982 Tau Beta Pi, Sigma Xi Honor Societies; 1992 Whitaker Foundation Young Investigator Award; 1996 S. Reid Warren Award for Distinguished Teaching; 1997 NSF Career Award; 2001 American Society of Mechanical Engineers Richard Skalak Best Paper Award; 2006 Fellow of the American Institute of Medical and Biological Engineering; 2007 Assoc of Women in Science Elizabeth W. Bingham Award for mentoring; 2009 Ford Motor Company Award for Faculty Advising; 2009 Fellow of the American Society for Mechanical Engineers; Fellow of the Biomedical Engineering Society.

C. Selected peer-reviewed publications (in chronological order).
(Publications selected from 92 peer-reviewed publications)

1. Duhaime AC, **Margulies SS,** Durham SR O'Rourke MM, Golden JA ,Marwaha S, Raghupathi R. Maturation-dependent response of the piglet brain to scaled cortical impact J Neurosurgery. 93(3):455-62, 2000. PMID: 10969944
2. Raghupathi, R and **Margulies SS**. Traumatic axonal injury after closed head injury in the neonatal pig. J Neurotrauma 2002; 19:843-853. PMID: 12184854
3. Prange MT, Coats B, Duhaime AC, and **Margulies SS**. Anthropomorphic simulations of falls shakes, and inflicted impacts for infants. J Neurosurg. 2003, 99: 143-150. PMID: 12854757
4. Gefen A, Gefen N, Zhu Q, Raghupathi R, and **Margulies SS**. Age-Dependent changes in material properties of the brain and braincase of the rat. J Neurotrauma. 2003 20:1163-1177. PMID: 14651804
5. Raghupathi R, Mehr MF, Helfaer MA, and **Margulies SS.** Traumatic Axonal Injury is Exacerbated following Repetitive Closed Head Injury in the Neonatal Pig J Neurotrauma 2004; 21:307-316. PMID: 15115605
6. Arbogast KB, Margulies SS and CW Christian Initial Neurological Presentation in Young Children Sustaining Inflicted and Unintentional Fatal Head Injuries. Pediatrics 2005; 116: 180-184. PMID: 15995050
7. Zhu Q, Prange M., **Margulies SS.** Predicting Unconsciousness From a Pediatric Brain Injury Threshold. Developmental. Neurosci (Invited) 2006:28:388-395. PMID: 16943662
8. Levchakov A, Linder-Ganz E, Raghupathi R, **Margulies SS,** and A Gefen Computational Studies of Strain Exposures in Neonate and Mature Rat Brains During Closed Head Impact J Neurotrauma 2006:1570-1580. PMID: 17020491
9. Coats BS and **Margulies S**S. Material Properties of Human Infant Skull and Suture at High Rates. J Neurotrauma 2006: 23(8):1222-1232. PMID: 16928180
10. Ichord R., Naim M., Pollack A., Ibrahim N., Christian C, and **Margulies SS**. Hypoxic-Ischemic Injury Complicates Traumatic Brain Injury in Infants: The Role of Diffusion Weighted Imaging. J Neurotrauma (Invited) 2007: 24:106-118. PMID: 17263674
11. Friess SH, Ichord R, Owens K, Ralston J, Overall K, Smith C, Helfaer M, and **Margulies SS**. Neurobehavioral Functional Deficits Following Closed Head Injury in the Neonatal Pig. Exper Neurol 2007: 204:234-43. PMID: 17174304
12. Coats B and **Margulies SS**. Potential for Head Injuries in Infants from Low Height Falls. Journal of Neurosurgery - Pediatrics 2008 Nov, 2(5):321-30. (**Selected for Editorial Comment and journal cover**) PMID: 18976102
13. **Margulies SS**, Hicks, R and the Combination Therapies for TBI Workshop Leaders. Combination Therapies for Traumatic Brain Injury –Prospective Considerations. J. Neurotrauma 26:925-939. PMID: 19331514
14. Friess SH, Ichord R, Owens K, Ralston J, Ryall K, Helfaer M, Smith C, and **Margulies SS**. Repeated traumatic brain injury affects composite cognitive function in piglets. Journal of Neurotrauma 26:1111-1121. PMID: 19275468
15. Zhou C, Eucker S, Durduran T, Yu G, Ralston J, Friess, SH, Ichord RN, **Margulies SS** and Yodh AG. Diffuse Optical Monitoring of Hemodynamic Changes in Piglet Brain with Closed Head Injury. J Biomed Optics 2009: 14: 034015 (June 4, 2009). PMID: 19566308

D. Current Research Support:

R01-HL-57204 Margulies (PI) 7/01/97 – 4/30/11
National Institutes of Health/NHLBI
Mechanical Injury of the Alveolar Epithelium
In this competitive renewal our major objective is to determine specific stretch-induced mechanical and molecular signals that modulate alveolar epithelial permeability during clinically relevant conditions - including chronic continuous cycling and ARDS
Role: PI

R01-NS-39679 Margulies (PI) 12/01/99 – 3/31/12
NIH- National Institute for Neurological Disorders and Stroke
Biomechanics of Pediatric Head Injury
In this competitive renewal our major objective is to determine mechanisms of primary and secondary brain injury in children, with an emphasis on both mild and severe injuries. The influences of rotation direction, hypoxia/ischemia, and repeated injuries will be studied using animal experiments and computational models.
Role: PI

R01-CE-001445 Margulies (PI) 9/30/08 – 9/30/11
Center for Disease Control, NCIPC
Development and Validation of a Diagnostic Tool for Infant Head Injuries from Falls
Biomechanical loads from surrogate experiments, pediatric large animal TBI data, and a computational model will be combined to create a predictive tool to determine the plausibility of skull fracture and extra-axial hemorrhages in infants following low height falls. Validated with real-word clinical data, this biomechanical data will advance the understanding of injury thresholds in common non- inflicted scenarios that will ultimately improve the accuracy in detection of inflicted and non-inflicted head trauma.
Role: PI

DTNH22-07-H-00088 Margulies (PI) 6/1/07-5/30/10
Department of Transportation - NHTSA
Brain Injury Criteria for 6 to 10 year old Children
This new project utilizes human accident reconstructions, 2-month old porcine inertial brain injury studies, and finite element modeling to identify injury thresholds for school-age children.
Role: PI

R01-NS-055951 Bayly (PI) 1/01/07 – 12/30/11
National Institutes of Health via subcontract from Washington University
In vivo Measurement of Brain Biomechanics
This project involves the use of brain-skull motion studies and brain property elastography studies in the measurement of the biomechanics of the brain.
Role: Investigator

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Mueller, Patrick J.	Assistant Professor
eRA COMMONS USER NAME	
MUELLERP	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Blackburn University, Carlinville, Illinois	B.A.	1990	Biology/Chemistry
St. Louis University, St. Louis, Missouri	Ph.D.	1996	Pharmacol. & Physiol.
Medical College of Wisconsin, Milwaukee, WI	Postdoc	1995-1997	Exerc Phys./Neural Ctrl.
University of Missouri, Columbia, MO	Postdoc	1997-2001	Neural Ctrl. Circulation

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. My laboratory has extensive experience in assessing hemodynamics under various conditions. Therefore, I will aide in the interpretation of all hemodynamic data. Further, if necessary, I will contribute my time to aide Dr. Kreipke in assessing whether his drug, Clazosentan, has effects on peripheral perfusion.

B. Positions and Honors.

Positions and Employment

1990-1995	Graduate Trainee, St. Louis University Health Sciences Center
1995-1997	Postdoctoral Fellow, Medical College of Wisconsin
1997-2001	Postdoctoral Fellow, University of Missouri-Columbia (MU)
2001-2/2007	Research Assistant Professor, Dalton Cardiovascular Research Center, MU
2003-2/2007	Research Investigator, Dalton Cardiovascular Research Center, MU
2003-2/2007	Adjunct Research Assistant Professor, Department of Biomedical Sciences, MU
3/2007-	Assistant Professor, Department of Physiology, Wayne State University School of Medicine
8/2007-	Graduate Faculty, Wayne State University School of Medicine

Honors

1989	Bonnie Keith Albracht Scholarship, Blackburn College, Carlinville IL
1989	C.H.C. Anderson Prize, Blackburn College, Carlinville IL
1989	Drew Thurston Memorial Award, Blackburn College, Carlinville IL
2001	Caroline tum Suden/Frances A. Hellebrandt Professional Opportunity Award, American Physiological Society, Experimental Biology Meeting
2001	Michael J. Brody Young Investigator Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2001	Phi Zeta Research Day, 1st Place Oral Presentation, Advanced Graduate Students and

	Postdocs
2005	Research Recognition Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2006	Research Career Enhancement Award, American Physiological Society Host Laboratory: Patrice Guyenet, Ph.D., University of Virginia
2007	Outstanding Poster picked for Oral Presentation FASEB Summer Research Conference: Sydney, Australia Neural Mechanisms in Cardiovascular Regulation
2009	New Investigator Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2009	Travel Award and Invited Speaker, International Society of Autonomic Neuroscience Satellite Meeting "Autonomic Adjustments to Environmental Challenges" Newcastle, Australia

Other Experience and Professional Memberships

Societies: American Physiological Society, Society for Neuroscience, American College of Sports Medicine

Reviewer: Am J Physiol: Heart Circ Physiol; Am J Physiol: Reg Integr Physiol; Autonom Neurosci: Basic and Clin; J Appl Physiol; Med Sci Sports & Exercise, Hypertension, Exp Physiol, BMC Neurosci. Univ of Florida Mock Grant Review (03/2004); AHA National Consortium Peer Review Committee (10/07).

Related Activities: Faculty Grant Writing Institute-University of Missouri-Columbia (05/06); Lecturer, Univ. of Missouri-Columbia, Biomed. Sci. Course VBSCI9467 "Neural Control of the Circulation" (Spring '02,'04,'06); Instructor, Central Neural Control of the Circulation, The American Physiological Society Latin American Initiative, Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo-Brazil (08/04); Poster Judge, Cardiovascular Day, Univ of Missouri (02/04), Minority Research Day, WSU (08/07) ; Architect Advisory Committee-Dalton Cardiovascular Research Center Expansion and Renovation Project (2001-2003); APS Committee Member, Neural Control and Autonomic Regulation Steering Committee, Member in Training (2001-2002); Lecturer, Wayne State University School of Medicine, Dept of Physiol PSL7600 "Advanced Cardiovascular Physiology" (Fall 2007-09); Laboratory Instructor, Medical Physiology, Wayne State University School of Medicine (Fall 2007-09).

C. Peer-reviewed Publications (selected from 26 peer reviewed works and 2 book chapters) .

1. **Mueller, P.J**. and Knuepfer, M.M. Coronary vascular effects of cocaine in rats. J Pharmacol Exp Ther 268: 97-103, 1994. PMID: 8301600
2. **Mueller, P.J.**, Gan, Q. and Knuepfer, M.M. Ethanol alters hemodynamic responses to cocaine in rats. *Drug Alcohol Depend* 48:17-24, 1997. PMID: 9330917.
3. Buckwalter, J.B., **Mueller, P.J.** and Clifford, P.S. Autonomic control of skeletal muscle vasodilation during exercise. *J Appl Physiol* 83 (6): 2037-2042, 1997. PMID: 9390978. http://jap.physiology.org/cgi/reprint/83/6/2037
4. Buckwalter, J.B., **Mueller, P.J.** and Clifford, P.S. Sympathetic vasoconstriction to active skeletal muscles during dynamic exercise. *J Appl Physiol* 83 (5):1575-80, 1997. PMID: 9375322. http://jap.physiology.org/cgi/reprint/83/5/1575
5. Buckwalter, J.B., Ruble, S.B., **Mueller, P.J.** and Clifford, P.S. Skeletal muscle vasodilation at the onset of exercise. *J Appl Physiol* 85 (5): 1649-1654, 1998. PMID: 9804565. http://jap.physiology.org/cgi/reprint/85/5/1649
6. **Mueller, P.J.**, O'Hagan, K.P., Skogg, K.A., Buckwalter, J.B. and Clifford, P.S. Renal hemodynamic responses to dynamic exercise in rabbits. *J Appl Physiol* 85 (5): 1605-1614, 1998. PMID: 9804559. http://jap.physiology.org/cgi/reprint/85/5/1605
7. Knuepfer, M.M., Gan, Q. and **Mueller, P.J.** Mechanisms of hemodynamic responses to cocaine in conscious rats. *J Cardiovasc Pharmacol* 31:391-399, 1998. PMID: 9514184.
8. **Mueller, P.J.** and Hasser, E.M. Enhanced sympathoinhibitory response to volume expansion in conscious hindlimb unloaded rats. *J. Appl. Physiol.* 94: 1806-1812, 2003. PMID: 12533501. http://jap.physiology.org/cgi/reprint/94/5/1806

9. **Mueller, P.J.**, Buckwalter, J.B. and Clifford, P.S. Tracheal tone and the role of ionotropic glutamate receptors in the nucleus ambiguus. *Brain Research* 1021: 54-62, 2004. PMID: 15328031.
10. **Mueller, P.J.**, Foley, C.M., and Hasser, E.M. Hindlimb unloading alters nitric oxide and autonomic control of resting arterial pressure in conscious rats. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 289: R140-R147, 2005. PMID: 15761183. http://ajpregu.physiology.org/cgi/reprint/289/1/R140
11. **Mueller, P.J.**, Sullivan, M.J., Grindstaff, R.R., Cunningham, T.J. and Hasser, E.M. Regulation of plasma vasopressin and renin activity in conscious hindlimb-unloaded rats. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 291: R46-R52, 2006. PMID: 16469838. http://ajpregu.physiology.org/cgi/reprint/291/1/R46
12. **Mueller, P.J.** Exercise training and sympathetic nervous system activity: Evidence for physical activity dependent plasticity. *J. Clin. Exp. Pharmacol. Physiol.* 34(4):377-84, 2007. PMID: 17324153.
13. **Mueller, P.J.** Influence of sedentary versus physically active conditions on regulation of plasma renin activity and vasopressin. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 295: R727-R732, 2008. PMID: 18509102. http://ajpregu.physiology.org/cgi/reprint/295/3/R727
14. Heesch, C.M., Foley, C.M., **Mueller, P.J.**, Hasser, E.M. and Patel, K.P. Nitric oxide synthase activity and expression are decreased in the paraventricular nucleus of pregnant rats. *Brain Res* 1251:140-50, 2009. PMID: 19041855
15. Austgen, J.R., Fong, A.Y., Foley, C.M., **Mueller, P.J.**, Heesch, C.M. and Hasser, E.M. Expression of group I metabotropic glutamate receptors on phenotypically different cells within the nucleus of the solitary tract in the rat. *Neuroscience* 159: 701-716, 2009. PMID: 19013221

D. Research Support

Ongoing Research Support

HL089364 — Mueller (PI) — 08/10/07-07/31/10
R21, National Institutes of Health
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
Role: PI

American Heart Association, Predoctoral Fellowship — Mischel (PI) — 07/09-06/11
"NMDA Receptor Neuroplasticity in the RVLM Following Imposition of Sedentary Conditions."
Role: Sponsor

R01, National Institutes of Health, — Kreipke (PI) — 07/01/09-06/30/14
"Molecular Basis of Enhanced Contractility after Traumatic Brain Injury: Towards a clinical Trial"
Role: Co-Investigator (10%)

New Faculty Startup Funds — Mueller (PI) — 03/01/07-Present
Wayne State University School of Medicine

Pending Research Support

R01, National Institutes of Health — Mueller (PI) — 07/01/10-06/30/15 (Pending)
"Inactivity and Enhanced Sympathoexcitation: Role of Neuroplasticity in the RVLM"
Role: PI

Research Support Completed During the Last Three Years

0650161Z* Mueller (PI) — 01/01/06-12/31/08*
Grant In Aid, American Heart Association, Heartland Affiliate
"Central Control of Sympathetic Outflow Following Exercise Training"

The major goals of this project are to 1) Examine the effect of ExTr on activation of SNS activity and spinally projecting RVLM neurons. 2) Examine the effect of ExTr on regulation of SNS outflow by altering tonic excitatory and inhibitory neurotransmission in the RVLM.
Role: PI

*Transferred to Wayne State University School of Medicine from University of Missouri 03-01-07.

HL089364 Mueller (PI) 08/10/07-05/19/08
R21, National Institutes of Health Supplement 2
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
This supplement supported a minority undergraduate student who worked in my laboratory - Jason Franco

HL089364 Mueller (PI) 08/10/07-07/31/09
R21, National Institutes of Health (HL089364) Supplement 1
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
This supplement supported a minority undergraduate student who worked in my laboratory - Janet Adedokun

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jose A. Rafols	Professor
eRA COMMONS USER NAME	
JOSERAFOLS	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Illinois Benedictine, Lisle, IL	B.S.	1965	Biology
University of Kansas, Kansas City, KS	Ph.D.	1969	Anatomy
S. Ramon Y Cajal Institute, CSIC, Madrid, Spain	Post Doc	1970	Neuroanatomy

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has been primarily interested in studying the effects of endothelin-1 and its receptors on microvascular tone following TBI and in stroke. Work completed in my laboratory includes data which emphasizes the differential roles that ETrA and B play on control of microvascular tone. This proposal, therefore, draws on this preclinical data to develop the most effective therapy to ameliorate hypoperfusion following TBI.

B. Positions and Honors

Positions and Employment

1969-1970	Instructor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1970	NIH Postdoctoral trainee at S. Ramon Y Cajal Institute, CSIC, Madrid, Spain
1971-1973	Asst. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1973-1989	Assoc. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1989-present	Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1994-present	Dir., Morphology and Imaging Core, Neurotrauma Center, Wayne State University, School of Medicine

Honors

DHHS/PHS/NIH Study Section Member (full member), Neurological Disorder Program Project Review A Committee (NSP-term) 7/1/90-6/30/94.
Chairman, Site visit, The Johns Hopkins University, Baltimore, MD; "Disorders of aging neuro-transmitter systems and neurotrophins", December 15-17, 1991.
Member, National Institutes of Health Reviewers Reserve (NRR), for term 7/1/94-6/30/98.
Member, American Heart Association National Study Committee, Brain Review Committee, for term 7/96-6/99.

C. Selected peer-reviewed publications (selected from 136 manuscripts)

Dore-Duffy P, Owen C, Bahabanov R, Murphy S, Rafols JA. 2000 Pericyte response to traumatic brain injury (TBI): Elongation and migration from the microvascular wall. Microvascular Res 60:55-69.

White BC, Sullivan JM, DeGracia DJ, O'Neill BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. 2000 Brain ischemia and reperfusion: Molecular mechanisms of neuronal injury. J Neurolog Sci 179:1-33.

Petrov T, Underwood W, Alousi S, Own C, Rafols JA. 2001 Upregulation of iNOS and eIF2άP expression is paralleled by suppression of protein synthesis in the hypothalamus following trauma to the brain. J Neurotrauma 18:799-812.

Petrov T and Rafols JA. 2001 Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. Neurol Res 23:138-143.

Petrov T, Steiner J, Braun B, Rafols JA. 2002 Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. Neurosci 115:275-283.

Petrov T, Rafols JA, Alousi SS, Kupsky WJ, Johnson R, Shah J, Shah A, Watson C. 2003 Cellular compartmentalization of Phosphorylated eIF2 alpha and neuronal NOS in human temporal lobe Epilepsy with hippocampal sclerosis. J Neurol Sci 209:31-39.

Steiner J, Rafols D, Park H, Katar MS, Rafols JA, Petrov T. 2004 Attenuation of inOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma. Nitric Oxide 10:162-169.

Rafols D, Steiner J, Rafols JA, Petrov T. 2004 Coexpression of iNOS and endothelin-1 mRNAs in specific cell types following traumatic brain injury. Neurosci letters 362:154-157.

Kreipke C, Rafols J, Petrov T. 2005. Transcriptional and translational mechanisms for the reciprocal control of iNOS and endothelin 1 expression in brain microvessels after traumatic brain injury (TBI). Journal of Cerebral Blood Flow and Metabolism 25, S191.

Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research. 71:197-204.

Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurol Res. 29:604-609.

Kallukuri S, Kreipke C, Rossi NF., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma Endothelin receptor localization following traumatic brain injury. Neurol Res 29:362-368.

Kreipke C, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurol Res 29:369-374.

Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.

Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.

D. Ongoing Research Support

R01 NS39860 J Rafols (PI) 3/10/04-4/30/10 3.6 calendar months
NIH-NINDS $230,000
"Control of microvascular tone in traumatic brain injury"
The major goal of the project is to Investigate the role of endothelin receptors in the control of the microcirculation in a rat model of traumatic brain injury.

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14 1.8 calendar months
NIH_NINDS $233,000
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial"
Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI.

VA RR&D Award N Rossi (PI) 1/01/08-12/31/11 3.6 calendar months
VA Rehabilitation Award $139,000
"Conditioning, microvascular tone & rehabilitation"
Investigates the role of exercise in controlling microcirculation after traumatic brain injury.

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Goshgarian, Harry G.	Professor of Anatomy/Cell Biology
eRA COMMONS USER NAME (credential, e.g., agency login) aa0845	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Massachusetts	B.S.	1971	Zoology
University of Michigan	M.S.	1973	Anatomy
University of Michigan	Ph.D.	1975	Anatomy
Section on Neural Development/Regeneration, Lab of Neurochemistry, National Institutes of Health	Postdoc	1975-1977	Neurochemistry

A. Personal Statement

I have been heavily funded to study plasticity and functional outcome following spinal cord injury. Further, my studies have focused on novel pharmacotherapies for improving respiratory drive following injury. The current proposal seeks to demonstrate an improvement in functional outcome following treatment of an endothelin-1 receptor A compound in two different models of TBI (rat and porcine). Given my background in both CNS injury models and pharmacological development I feel that I am well suited to act as a consultant for this project should any conflict arise in interpretation of the results that Drs. Kreipke and Armstead collect.

B. Positions and Honors.

Positions and Employment:

08/01/77 – 08/31/82 Assistant Professor of Anatomy, Wayne State University
09/01/81 – 08/31/90 Associate Professor of Anatomy, Wayne State University
10/11/84 – present Associate in Orthopedic Surgery, Wayne State University
09/01/90 – present Professor of Anatomy/Cell Biology, Wayne State University
06/01/2000 – present Associate in Internal Medicine, Wayne State University

Honors:

NIH MERIT AWARD (NICHD), 1999-2009

C. Selected peer-reviewed publications (from a list of 85 publications):

1. Phillis, J.W. and **Goshgarian, H.G**. Adenosine and neurotrauma: therapeutic perspectives. *Neurol. Res.* 23(2-3): 183-189, 2001. PMID: 11320597
2. Nantwi, K.D. and **Goshgarian, H.G**. Alkylxanthine-induced recovery of respiratory function following cervical spinal cord injury in adult rats. *Exp. Neurol.* 168(1):123-134, 2001. PMID: 11170727 doi:10.1006/exnr.2000.7581
3. Basura, G.J., Nantwi, K.D., and **Goshgarian, H.G**. Theophylline-induced respiratory recovery following cervical spinal cord hemisection is augmented by serotonin 2 receptor stimulation. *Brain Res.* 956(1): 1-13, 2002. PMID: 12426040 http://www3.interscience.wiley.com/cgi-bin/fulltext/118951700/PDFSTART
4. Nantwi, K.D. and **Goshgarian, H.G**. Actions of specific adenosine receptor A1 and A2 agonists and antagonists in recovery of phrenic motor output following upper cervical spinal cord injury in adult rats. *Clin. Exp. Pharmacol. Physiol.* 29(10): 915-923, 2002. PMID: 12207572 http://www3.interscience.wiley.com/cgi-bin/fulltext/118951700/PDFSTART
5. Nantwi, K.D., Basura, G.J., and **Goshgarian, H.G**. Effects of long-term theophylline exposure on recovery of respiratory function and expression of adenosine A1 mRNA in cervical spinal cord

hemisected adult rats. *Exp. Neurol.* 182(1): 232-239, 2003. PMID: 12821393 doi:10.1016/S0014-4886(03)00109-2

6. **Goshgarian, H.G.** The crossed phrenic phenomenon: a model for plasticity in the respiratory pathways following spinal cord injury. *J. Appl. Physiol.* 94(2): 795-810, 2003. PMID: 12531916 http://jap.physiology.org/cgi/reprint/94/2/795
7. Bascom, A.T., Lattin, C.D., Aboussouan, L.S., and **Goshgarian, H.G**. Effect of acute aminophylline administration on diaphragm function in high cervical tetraplegia: a case report. *Chest* 127(2): 658-661, 2005. PMID: 15706011. http://www.chestjournal.org/cgi/reprint/127/2/658
8. Bae, H., Nantwi, K,D., and **Goshgarian, H.G**. Recovery of respiratory function following C2 hemi and carotid body denervation in adult rats: influence of peripheral adenosine receptors. *Exp. Neurol.* 191(1): 94-103, 2005. PMID: 15589516 doi:10.1016/j.expneurol.2004
9. Tzelepis, G.E., Bascom, A.T., Badr, S.M., and **Goshgarian, H.G**. Effects of theophylline on pulmonary function in patients with traumatic tetraplegia. *J. Spinal Cord Med.* 29(3): 227-233, 2006. PMID: 16859226 http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1864809&blobtype=pdf
10. Alilain, W.J. and **Goshgarian, H.G.** MK-801 upregulates NR2A protein levels and induces functional recovery of the ipsilateral hemidiaphragm following acute C2 hemisection in adult rats. *J. Spinal Cord Med.* 30(4): 346-354, 2007. PMID 17853656. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=2031932&blobtype=pdf
11. Kajana, S. and **Goshgarian, H.G**. Administration of phosphodiesterase inhibitors and an adenosine A1 receptor antagonist induces phrenic nerve recovery in high cervical spinal cord injured rats. *Exp. Neurol.* 210(2): 671-680, 2008. PMID: 18289533 doi:10.1016/j.expneurol.2007.12.021 .
12. Alilain, W.J, and **Goshgarian, H.G.** Glutamate receptor plasticity and activity-regulated cytoskeletal associated protein regulation in the phrenic motor nucleus may mediate spontaneous recovery of the hemidiaphragm following chronic cervical spinal cord injury. *Exp Neurol.* 212(2): 348-357, 2008. PMID: 18534577 doi:10.1016/j.expneurol.2008.
13. Kajana, S. and **Goshgarian, H.G**. Spinal activation of the cAMP-PKA pathway induces respiratory motor recovery following high cervical spinal cord injury. *Brain Res.* 1232:206-13, 2008. PMID: 18656458. doi:10.1016/j.brainres.2008.07
14. Kajana, S. and **Goshgarian, H.G.** Systemic administration of rolipram increases medullary and spinal cAMP and activates a latent respiratory motor pathway after high cervical spinal cord injury. *J Spinal Cord Med.* 32(2):175-82, 2009. PMID: 19569465.
15. Huang, Y. and **Goshgarian, H.G.** Postnatal conversion of cross phrenic activity from an active to latent state. *Exp Neurol.* 219(1):66-73, 2009. PMID: 19416665.

D. Research Support.

Ongoing Research Support:

R37 HD31550 Goshgarian (PI) 07/04/04-05/31/10
NIH/NICHD
Functional Plasticity in the Mammalian Spinal Cord
The above is the second five year cycle of a 10 year NIH MERIT Award whose goals are to understand the underlying mechanisms related to recovery of respiratory muscles paralyzed by cervical spinal cord injury. Several of the techniques pertaining to the plasticity of glutamate receptors and their subunits proposed in this application are routine in my laboratory and are funded by this grant.
Role: PI

There is no other support available to the PI.

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME	POSITION TITLE
Smith, Douglas H.	Professor of Neurosurgery
eRA COMMONS USER NAME (credential, e.g., agency login) smithdou	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Connecticut	B.S.	05/1981	Biology
University of Noreste	M.D.	05/1986	Medicine
University of Connecticut Health Center	Fellow	1986-1988	Biochemistry
University of Connecticut Health Center	Fellow	1988-1990	Neurotrauma

A. Personal Statement

Dr. Smith has over 18 years experience with brain injury research and has collaborated with the PI, Dr. Armstead, over several years. In particular, Dr. Smith's group has extensive expertise with traumatic brain injury in swine, examining histopathological outcome.

B. Positions and Honors

Positions and Employment

1986-88 Postdoctoral Fellow in Protein Chemistry and Molecular Biology, Dept. of Biochemistry, University of Connecticut Health Center, Farmington, CT
1989-90 Postdoctoral Fellow in Neurotrauma and Neuropharmacology, Dept. of Surgery, University of Connecticut Health Center, Farmington, CT
1991-92 Assistant Professor, Surgical Research Center, Dept. of Surgery, Univ. of Conn. Health Center
1992-97 Assistant Professor, Dept. of Neurosurgery, University of Pennsylvania, Philadelphia, PA
1995-01 Associate Director, Center for Brain Injury and Repair, University of Penn., Philadelphia, PA
1998-03 Associate Professor, Department of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA
2004- Professor, Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA
2004- Director, Center for Brain Injury and Repair, University of Pennsylvania, Philadelphia, PA
2009- Vice-Chairman for Research, Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA

Honors

1992 University of Connecticut Health Center Research Advisory Committee Faculty Award
1993 Young Scientist Award, 2nd International Neurotrauma Symposium, Glasgow, Scotland
1994 Brain Trauma Foundation Research Award
1995- Member, Editorial Board, Journal of Neurotrauma
2000-03 Member, Brain Disorders and Cognitive Neuroscience-3 (BDCN-3) Study Section, National Institutes of Health
2003-04 Vice-President, National Neurotrauma Society
2006-09 Councilor, National Neurotrauma Society
2009- Robert A. Groff Endowed Professor of Neurosurgery, University of Pennsylvania, Philadelphia, PA

C. Selected Peer-reviewed Publications (Selected from over 130 peer-reviewed publications

Most relevant to the current application (5)

Armstead WM, Nassar T, Akkawi S, **Smith DH**, Chen X-H, Cines DB, Higazi AA. Neutralizing the neurotoxic effects of exogenous and endogenous tPA. *Nature Neurosci.* 9(9);1150-1155, 2006.

Armstead WM, Ganguly K, Kiessling JW, Chen XH, **Smith DH**, Higazi AR, Cines DB, Bdeir K, Zaitsey S, Muzykantoy VR. RBC-coupled tPA prevents impairment of cerebral vasodilatory responses and tissue injury

in pediatric cerebral hypoxia/ischemia through inhibition of ERK MAPK activation. *JCBFM*. 29(8):1463-1474, 2009.

Zhang J, Groff R, Chen X-H, Browne KD, Huang J, Schwartz ED, Meaney DF, Johnson VE, Stein SC, Rojkjaer R, **Smith DH**. Hemostatic and neuroprotective effects of human recombinant activated factor VII therapy after traumatic brain injury in pigs. *Exp. Neurol.*, 210(2):645-55, 2008.

Chen XH, Meaney DF, Xu BN, Nonaka M, McIntosh TK, Wolf JA, Saatman KE, **Smith DH**. Evolution of neurofilament subtype accumulation in axons following diffuse brain injury in the pig. *J Neuropathol Exp Neurol.* 58:588-596, 1999.

Smith DH, Chen XH, Nonaka M, Trojanowski JQ, Lee VMY, Saatman KE, Leoni MJ, Xu B-N, Wolf JA, Meaney DF. Accumulation of Amyloid β and Tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. *J Neuropathol Exp Neurol.* 58:982-992, 1999.

Additional recent publications of importance to the field (in chronological order) (10)

Smith DH, Meaney DF. Axonal damage in traumatic brain injury. *The Neuroscientist* 6(6) 483-495, 2000.

Chen X-H, Siman R, Iwata A, Meaney DF, Trojanowski JQ, **Smith DH**. Long-term accumulation of Amyloid-ß, ß-secretase, Presenilin-1, and Caspase-3 in damaged axons following brain trauma. *Am. J. Pathol.* 165(2):357-371, 2004.

Smith DH, Nonaka M, Miller R, Leoni M, Chen X-H, Alsop D, Meaney DF. Immediate coma following inertial brain injury in dependent on axonal pathology in the brain stem. *J Neurosurgery*, 93:315-322, 2000.

Wolf JA, Stys PK, Lusardi T, Meaney DF, **Smith DH.** Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. *J Neurosci.* 21 (6): 1923-1930, 2001.

Smith DH, Meaney DF, Shull WS. Diffuse axonal injury in head trauma. *J Head Trauma Rehab.* 18(4) 307-316, 2003.

Smith DH, Chen X-H, Iwata A, Graham DI. Amyloid-ß in axons after traumatic brain injury in humans. *J Neurosurg.* 98:1072-1077, 2003.

Smith DH, Uryu K, Saatman KE, Trojanowski JQ, McIntosh TK. Protein accumulation in traumatic brain injury. *Neuro Molecular Med.* 4(1-2):59-72, 2003.

Iwata A, Stys PK, Wolf JA, Chen X-H, Taylor AG, Meaney DF, **Smith DH**. Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. *J Neurosci.* 24(19) 4605-4613, 2004.

Uryu K, Chen X-H, Martinez D, Browne KD, Johnson VE, Graham DI, Lee VM-Y, Trojanowski JQ, **Smith DH**. Multiple proteins limplicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. *Exp. Neurology* 208(2): 185-92, 2007.

Chen X-H, Johnson VE, Uryu K, Trojanowski JQ, **Smith DH**. A lack of amyloid β plaques despite persistent accumulation of amyloid β in axons of long-term survivors of traumatic brain injury. *Brain Pathology* 19(2):2214-23, 2009.

D. Research Support – from last 3 years

Ongoing Research Support

R01 NS048949 Smith 03/01/2006 – 02/28/2010
NIH NINDS
Spinal Cord Repair with Nerve Constructs. The key aims are to use engineered nervous tissue constructs to repair spinal cord injury. The efficacy of this treatment will be evaluated in rat spinal cord injury models of lateral hemisection of the thoracic spinal cord and complete transection of the thoracic spinal cord. Role: PI

T32-NS043126 Smith 07/01/2008 - 06/30/2013
NIH NINDS
Brain Injury Training Grant. The principal goal of the brain injury training grant is to provide an excellent mentoring environment for M.D. and Ph.D. trainees to prepare them for careers in nervous system injury research. Our trainees acquire basic science research skills that address the etiology, pathogenesis, diagnosis, treatment, and prevention of injury to the nervous system, such as traumatic brain injury (TBI) and cerebral ischemia (stroke). Role: PI

P01-NS056202 Smith 08/01/2008 - 07/31/2013
NIH
Mild Traumatic Brain Injury and Diffuse Axonal Injury. The overall program project seeks to understand the specific cellular and molecular mechanisms underlying cell death and dysfunction to further the development of therapeutic strategies targeted to treat human brain injury. Role: PI

R01 HL077760 Higazi 04/01/2006 - 03/31/2010
NIH NHLBI
tPA in traumatic brain injury. This project evaluates the protective and deleterious roles of tPA in models of traumatic brain injury. Role: Co-I

1-R01-NS-053410-01A1 Armstead 06/01/2006-05/31/2011
National Institutes of Health
Plasminogen activators and cerebral ischemic injury.

Completed Research Support

R01 NS038104 Smith 12/15/2003 - 01/31/2010 No Cost Extension Yr
NIH NINDS
Pathophysiology of Traumatic Axonal Injury. The goal of this project is to evaluate mechanisms of amyloid-ß accumulation in damaged axons and deposition in the brain following traumatic brain injury. The key aim of this grant is to elucidate the mechanistic link between a history of traumatic brain injury and an increased risk of developing Alzheimer's disease (AD), by identifying shared pathologic pathways. Role: PI

n/a Smith 01/01/2008-12/31/2008
Nanotechnology Institute of Philadelphia
Blast Injury Dosimeter Development. Role: PI

n/a Cassacia-Bonnefil 04/16/2007-04/16/2009
New Jersey Center for Brain Injury & Repair
Molecular mechanisms of delayed axonal damage in traumatic brain injury. Role: PI

n/a PI?? 12/15/2006-12/15/2007
Second Sight of New York
Evaluation of culture environmental effects of ECP strand properties and the interface of cultures neuronal cells to ECP strands.. Role: Co-Investigator

n/a Smith 01/01/2006-06/01/2009
Novo-Nordisk
Mechanisms of neuroprotective effects of Novo 7 after traumatic brain injury. Role: PI

RESOURCES—WAYNE STATE UNIVERSITY

Follow the 398 application instructions in Part I, 2.7 Resources.

Year 1 Rat TBI studies (Wayne State University)

Office Space

Dr. Kreipke has a 300 sq ft office which is entirely dedicated to him. He has 700 sq ft additional space for technicians, postdoctoral fellows, and graduate students. His office contains one computer while the additional office space houses two desktops and two laptops. Each Co-I has his/her own office with computer.

Animal Housing and Care

Facilities for the care and housing of experimental animals are available in the basement of Scott Hall. These resources are operated by the University Department of Laboratory Animal Resources. At WSU, all animals used for biomedical research at the medical center are housed in modern animal care facilities with excellent supervisory and veterinary support.

Surgery

Dr. Kreipke currently manages a 700 sq. ft. laboratory (9320 Scott Hall) fully IACUC/DLAR approved for rodent surgery. All necessary tools for surgery are currently available to his laboratory. A modest request for maintaining such supplies (sutures, scalpels, gauze, cotton, replacement hair clipper blades, etc.) is included in the budget. In addition, Dr. Kreipke also has a 400 sq ft. facility for post-operative care, housing and maintenance which is also fully IACUC/DLAR approved.

CBF determination

Wayne State University, in association with Harper Hospital, houses a MRI facility core which contains the 4.7 T Brucker magnet fully equipped which arterial spin labeling software which will be used for all determination of CBF. While this is a shared magnet, the addition of a new 7.0 T magnet has greatly decreased the need for usage of the 4.7 T, allowing full access for Dr. Kreipke and his team.

Behavioral suite

Dr. Kreipke manages a 400 sq ft. (9332 Scott Hall) behavioral core which presently includes two radial arm mazes which are fully automated using Smart™ Version 2.5 software (San Diego Instruments, San Diego, CA). A modest request for two additional radial arm mazes is included in the budget.

RESOURCES—UNIVERSITY OF PENNSYLVANIA

Follow the 398 application instructions in Part I, 2.7 Resources.

Years 2 and 3 Porcine TBI studies (University of Pennsylvania)

Office Space

Dr. Armstead has a 100 sq ft office dedicated entirely to him with a desktop computer. His office is connected to his laboratory. Co-Is each have their own office with computers available to them.

Animal Housing and Care

Pigs are purchased from a commercial breeder and are maintained in a University animal facility. A dedicated sterile surgical suite will be available for survival traumatic brain injury studies of pigs. These resources are operated by the University Department of Laboratory Animal Resources.

Surgery

Dr. Armstead has a 380 sq ft laboratory and currently possesses a lateral fluid percussion brain injury device (designed and built by Medical College of Virginia), small animal ventilators, Siemens 248 Blood Gas analyzer, balance, pumps, and surgical supplies. A modest request for a dedicated lateral fluid percussion brain injury device, anesthesia machine, and small animal ventilator is requested since it would be cumbersome and difficult to move on a continual rotating basis the current devices used for acute studies between the Armstead lab and a sterile surgical suite for survival studies.

CBF determination

The Center for Advanced Magnetic Resonance Imaging and Spectroscopy in the Department of Radiology currently operates several MRI scanners fully dedicated for research protocols. The scanner that will be used in the proposed porcine studies is a 3.0 Siemens TIM Trio. Gradient coils are capable of imaging at 40mT/m with slew rates in excess of 200 T/m/s. This scanner includes standard capabilities for echoplanar imaging, arterial spin labeled perfusion imaging, diffusion imaging, angiography, spectroscopy, and spectroscopic imaging. BOLD fMRI sequences include automatic higher order shimming and both prospective and retrospective motion correction. Gradient performance allows 4 mm isotropic voxels at TR=2 sec and 3 mm isotropic voxels at TR=3 sec. The 3T system has several transmit/receive volume head coils and a multi-element receive only volume coil suitable for parallel acquisition schemes. Image data from these scanners can be ported directly to CD or a local workstation for back-up onto DVD for larger data sets.
Data analysis will take place in the Center for Functional Imaging (CfN) (www.cfn.upenn.edu) which is a Type 1 Center within the Departments of Radiology and Neurology that provides infrastructure support for functional neuroimaging at the University of Pennsylvania. The CfN is comprised of investigators and staff with a broad range of expertise in neuroimaging including regulatory affairs, MRI methods development, MRI physics and pulse programming, instrumentation, experimental design, computing, and image analysis procedures.
The CfN data analysis cluster houses a total of 12 public workstations connected to 40+ CPUs running 32-bit x 86 (Linux) gigabit networking cluster. There is 40 terabyte online disk strorage in 3 RAID arrays to provide online storage, with backup via a 484-slot LTO Ultrium-3 tape library for periodic full backups and nightly incremental backups. Software to run a broad range of data preprocessing and analysis procedures is available, including VoxBo (developed at Penn), Matlab, IDL, SPM, FSL, AFNI, AIR, Brain Voyager, SNAP, FreeSurfer and others. Two rooms have a 42-inch plasma screen for meetings and presentations. A public (PennNet) server, including web service, mailing lists, ftp download, source code management, calendaring, database hosting and mediawiki-based wikis also serves the CfN community.

Behavioral Determination

Behavior studies will be performed in the Neurosurgical Trauma Laboratory located in the medical school complex, 3 blocks from Dr. Margulies' office in Hayden Hall and Injury Biomechanics lab in the Towne Building. There is a 45ft x 75ft x 24ft pen of 12 interlocking industrial plastic pieces that allows for behavior tests such as balance beam, open field, food cover, glass frustration and T-maze. Assessments are □recorded with a Philips DVDR 75, a dual voltage color video camera with 4mm lens, and a Panasonic 20 inch television.

Program Director/Principal Investigator (Last, First, Middle): kreipke, Christian W

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ RESUBMISSION of application number:
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number:
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number:
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.
Name of former program director/principal investigator:

☐ CHANGE of Grantee Institution. Name of former institution:

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

INVENTIONS AND PATENTS *(Renewal appl. only)* ☐ No ☐ Yes
If "Yes," ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	$0.00	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 08/25/08 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	686,015	x Rate applied	52.00	% = F&A costs	$	356,728
b. 02 year	Amount of base $	39,971	x Rate applied	52.00	% = F&A costs	$	20,785
c. 03 year	Amount of base $	41,170	x Rate applied	52.00	% = F&A costs	$	21,408
d. 04 year	Amount of base $	96,205	x Rate applied	52.00	% = F&A costs	$	50,027
e. 05 year	Amount of base $		x Rate applied		% = F&A costs	$	
					TOTAL F&A Costs	$	448,948

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☐ Yes ☐ No

A. Specific AIMS

Traumatic brain injury (TBI), the leading cause of death and disability amongst our youth (CDC, 2004) and the signature injury in the "War on Terrorism", is characterized by three major pathologies: 1) cerebral edema, 2) diffuse axonal injury (DAI), and 3) enhanced vasoreactivity leading to hypoperfusion of the brain's parenchyma. To date, over 25 clinical trials have been developed to improve outcome by addressing the first two pathologies. None have been successful (Povlishock, 2008). However, to our knowledge no one has developed studies to address the clinical relevance of the third pathology, hypoperfusion.

In 1996, Dr. Armstead published that endothelin-1 (ET-1), a powerful vasoconstrictor, plays a critical role in mediating vasoconstriction following TBI (Armstead, 1996). This work led to multiple studies that focused on the role of ET-1 and its receptors, ETrA (primarily mediates vasoconstriction) and B (primarily mediates vasodilation), in mitigating hypoperfusion following TBI (Sato and Noble, 1998; Zhang et al., 2000; Armstead, 2004; Kreipke et al., 2009). Several laboratories have published that blocking ETrA using BQ-123, a specific ETrA antagonist, improves cerebral blood flow (CBF) after TBI (reviewed in Armstead, 2004; Kreipke et al., 2009). Dr. Kreipke further studied the effects of hypoperfusion on cellular and behavioral outcome following TBI and showed that BQ-123 reduces the extent of hypoperfusion which, in turn, improves both cellular and behavioral outcome following TBI (Kreipke et al., 2009). While these results show promise, enthusiasm for clinical application of this work has been dampened by a lack of a clinically relevant drug that is specific to ETrA (e.g., Bosentan, a mixed ETrA/B antagonist, causes systemic hypotension and, thus, is not a suitable candidate for improving outcome following TBI). However, in 2007 Actelion developed Clazosentan, which is the most highly specific ETrA antagonist currently available (10-100X more selective than previously available drugs [Bosentan, Enrasentan, Tezosentan, Darusentan]) (Baltistini et al., 2004) and which is currently undergoing Phase III clinical trial for use after aneurysmal subarachnoid hemorrhage (SAH). While much pertinent information regarding toxicity, absorption, metabolism, etc. has already been gained, to date no one has tested the efficacy of Clazosentan following TBI. To this end, we have begun to conduct preliminary studies which show that Clazosentan recapitulates data gained from BQ-123 (e.g., a 1mg/kg dose administered at 2 h post TBI completely ameliorates hypoperfusion). Clazosentan has the clinical use advantage of possessing an activity profile utilizing intravenous dosing administration devoid of significant systemic peripheral vascular action. Since much of the Investigation of New Drug (IND) enabling studies have already been performed by Actelion in support of the SAH trial and will be shared with us by the company, our proposal will only consider dosing optimization relative to time of administration and time window of therapeutic outcome efficacy.

The **Goal** of this U01 proposal is to test whether **Clazosentan is effective in ameliorating hypoperfusion and, ultimately, improving behavioral outcome following TBI.** In order to accomplish this goal, Drs. Kreipke's and Armstead's teams will combine resources to investigate Clazosentan in two different models of diffuse brain injury, the rodent weight acceleration impact and porcine fluid percussion models. Since, mechanistically, we have already determined that improved CBF reduces cellular damage which then, in turn, improves behavior, we have, in consultation with Program at NINDS, streamlined this application to focus on blood flow and behavioral outcome. Therefore, as outcome measures, we will, in the same animals, measure both CBF using MRI imaging and behavioral outcome. Use of two different species and types of TBI in these studies will strengthen and support the broader applicability of the results to the human. The proposal paradigm will use the rat as a high throughput vehicle for establishing initial efficacy of drug, while the pig will be used to establish translational relevance. The target population in human TBI that will be modeled by our preclinical studies is one of moderate to severe injury that leads to spasm. The following Specific Aims have been developed to accomplish the above goal statement:

Specific AIMS

1). Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either 2 or 8 h post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a rodent model of TBI.

2). Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either 2 or 8 h post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a porcine model of TBI.

B. Background: Significance and Innovation

Traumatic Brain Injury (TBI) and Brain Pathology: Why focus on blood flow following injury?

TBI results in several major histopathologic events, including among others: cerebral edema which leads to a critical rise in intracranial pressure, diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. In closed head TBI incidents in humans, all these events are thought to significantly contribute to the ensuing morbidity and mortality encountered in clinical settings. Over 25 clinical trials have been developed aimed at reducing or treating the first two pathologies. None have proven successful in moving forward on a treatment paradigm. However, no one has initiated a clinical trial aimed at alleviating hypoperfusion following TBI. As reviewed in 2001, trauma-induced decreases in cerebral blood flow directly contribute to poor outcome in patients and that understanding management of CBF could be critical to improved outcome (Zwienenberg and Muizelaar, 2001). Therefore, our combined laboratories have dedicated the last decade to understanding changes in brain microcirculation following trauma. Both laboratories, using different models of TBI and different species (rat and porcine) have shown that TBI results in a significant reduction of CBF (Armstead 1996; Rafols et al., 2007). This coincides temporally with enhanced vascular stress response (as determined by HSP-70 immunocytochemistry), neuronal injury (Rafols et al., 2007), impairment of ATP-sensitive K+ channels (Armstead, 1999), suppressed cellular energy levels in neurons (Huttemann et al., 2007), and poor cognitive outcome (Kreipke et al., 2007). Taken together, these results suggest that hypoperfusion has a profound effect on outcome after TBI. Therefore, this proposal will seek to reduce the extent and duration of hypoperfusion in order to improve behavioral outcome following TBI.

Endothelin-1 (ET-1), its receptors, and control of CBF following TBI.

Endothelin-1 (ET-1) is a powerful vasoconstrictor which has been localized to brain endothelial cells, glia, and neurons (Rafols, 2007). Acting through its receptors, A (ETrA) and B (ETrB), ET-1 signals downstream molecular changes in smooth muscle (SM) of reacting microvessels, ultimately affecting SM's contractility and the cerebral microcirculation. ET-1, as well as a host of other vascular agents such as adenosine, prostaglandins and nitric oxide (NO) are thought to interact metabolically to modulate vasoconstriction and vasodilation, ultimately maintaining vascular tone in CBF autoregulation (Andersson, 2001). Metabolic disruption of the balance between these substances can lead to altered vascular tone and autoregulatory capacity, such as those seen after TBI (Rafols, 2007).

In peripheral vascular beds there is consensus in the literature regarding the role of ETrA in mediating vasoconstriction (reviewed in Jacobs et al. 2006). We have previously shown that brain ETrA is upregulated following TBI (Rafols, 2007). Attenuation of ETrA in brain resulted generally in an increase in the cross-sectional diameter of cerebral arteries, especially following subarachnoid hemorrhage (Ishikawa et al, 1992). Furthermore, in vivo blockade of ETrA with BQ-123 (a selective ETrA antagonist) caused a reduction of a focal ischemic lesion (Barone et al, 2000).

While ETrB has been also shown to be upregulated in brain following TBI (Rafols, 2007), its role in the control of cerebral microvascular tone is controversial. As such, in peripheral vascular beds, ETrB has been shown to mediate both vasoconstriction and vasodilation. In one study, for example, elevation of blood pressure induced by ET-1 application was eliminated by BQ-788, a selective ETrB anatagonist (Ishikawa et al., 1994). Several other studies, however, have shown that ETrB antagonism has no effect on blood pressure (reviewed in Pollack and Schneider, 2006). One study, in particular, showed that, in pial vessels, BQ-788 abolished BQ-3020 (a selective ETrB agonist)-induced vasodilation (Touzani et al., 1997).

Collectively, results from our laboratories suggest that altered ETrA expression may underlie dysfunctional control of microcirculation after TBI. Thus by blocking ETrA we were able to restore CBF to normal levels after trauma using BQ-123 (Kreipke et a., 2009). In contrast ETrB blockade did not ameliorate hypoperfusion after trauma (Kreipke et al., 2009). In fact, at 48 hours post injury, 20nmol BQ-788 further decreased CBF suggesting that it has a deleterious role in the control of vascular tone after injury. By blocking ETrB, more ET-1 may be available for ETrA, thus enhancing vasoconstriction. Similarly, ETrB may participate in mediating the hyperemia detected with ETrA antagonism. It is possible that ETrA

antagonism preferentially shifts ET-1 binding to the ETrB receptor which could yield increased vasodilation and, hence, hyperemia (Begnini, 2007). Taken together, these results suggest that pharmacological strategies aimed at selectively blocking ETrA with limited to no blockade of ETrB could show promise in improving outcome following TBI.

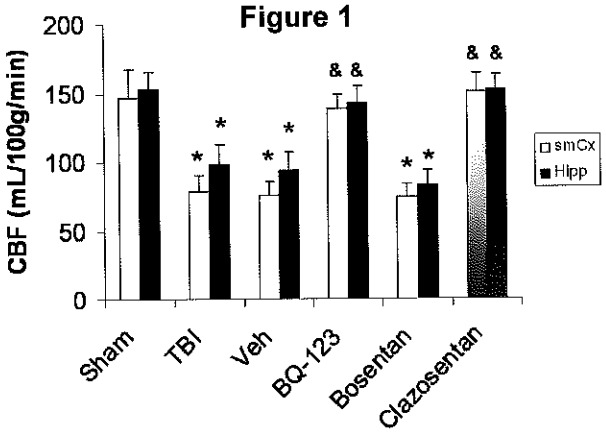
Innovation: Why use Clazosentan?

Brief history of development

In 1995 Luscher and Wenzel published one of the first reviews which characterized ET-antagonists as potential clinical therapeutics for vascular disorders (Luscher and Wenzel, 1995). In 1999, Benigni and Remuzzi published a follow-up which summarized data from pre-clinical and clinical studies which showed promise for specific ETrA antagonists in controlling hypertension. Bosentan, a mixed antagonist (ETrA and B) was discussed and clinical trial suggested that the potential opposing effects of ETrA and B may render Bosentan less effective (Benigni and Remuzzi, 1999). In 2003, it was reported that, after thorough investigation of ongoing clinical trial, Bosentan had some success in control of pulmonary arterial hypertension, however was not more effective than other, non-endothelial specific drugs (Krum and Liew, 2003). Once again, this may be attributed to Bosentan being a mixed antagonist. At the 2007 10th international symposium on endothelin (ET-10) in Bergamo, Italy, several investigators pointed out that while mixed antagonists have had some effects in pre-clinical studies, overall these agents have had little to no effect in the clinical setting. Therefore, it was proposed that more specific ETrA antagonists may be more useful.

The first report on a new drug, produced by Actelion Pharmaceuticals, INC in Switzerland, Ro 61-1790 [5-methyl-pyridine-2-sulfonic acid 6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2-(2-1H-tetrazol-5-yl-+ ++pyridin-4-yl)-pyrimidin-4-ylamide] was published in 1997 (Roux et al., 1997). It was found to be 1000-fold more selective for ETrA than ETrB. It was suggested that Ro 61-1790, which was renamed Clazosentan, may be useful for TBI (Sato and Noble, 1998), ischemia (Dawson et al., 1999), and subarachnoid hemorrhage (Gorlach et al., 2001). In 2006, clazosentan was included in a clinical trial to prevent vasospasm following hemorrhage (Uhlmann, 2006). Interestingly, this drug has been shown to have little effect in non-brain areas (Vuurmans et al., 2004). Further, as shown in ***Figure 1***, we have compared the effects of BQ-123 (a selective ETrA antagonist) with both Bosentan and Clazosentan on their ability to improve CBF following TBI (all drugs given at 30 min post TBI) and found that Clazosentan recapitulates the data seen by BQ-123 administration while Bosentan did not improve CBF. Therefore, Clazosentan provides a great potential for controlling hypoperfusion following TBI.

Figure 1



Established information on safety, toxicity, absorption, and metabolism:

Clazosentan is currently undergoing Phase III clinical trial for use after aneurysmal subarachnoid hemorrhage. Therefore, much of the pharmacokinetic and safety information has been already published. Further, due to the relationship between Dr. Kreipke and Actelion, other pertinent information will be included in the application for IND. The following is a brief summary of findings:

Early investigations (summarized in Vatter et al., 2005a,b) showed that, unique to all endothelin class drugs, Clazosentan had a strong affinity to ETrA (2000X more efficacious to ETrA than ETrB), which makes this a potentially superior drug over other "mixed" antagonists which block both vasoconstrictory and vasodilatory properties of brain vascularization. In an initial investigation of Clazosentan in humans, drug was infused at doses of 3-60 mg/h for 3h, 60 mg/h for 6 h and at 30 mg/h for 12h. Each dose was given to a separate group of subjects, six of whom received clazosentan and two placebo. Vital signs, ECG, adverse events, and clinical laboratory variables were monitored to assess tolerability. Blood and urine samples were collected frequently for pharmacokinetic and pharmacodynamic determinations.

Infusion of clazosentan up to doses of 30 mg/h for 3h was well tolerated. A dose of 60 mg/h and longer infusions were less well tolerated and three subjects did not complete the 12h infusion of 30 mg/h due to adverse events. Headache was the most commonly reported adverse event followed by nausea, vomiting, and nasal congestion. The pharmacokinetics of clazosentan was dose proportional in the dose range investigated. Values (mean and 95% confidence intervals) for clearance and volume of distribution at a dose of 10 mg/h for 3h were 42.2 (36.6, 48.7) l/h and 32.4 (27.0, 38.8) l, respectively. Both variables were independent of dose. The elimination of clazosentan was characterized by a very rapid disposition phase with a half-life of 6-10min. Compared to baseline, endothelin-1 concentrations increased approximately 2-fold after infusion of clazosentan but no dose-dependent relationship could be discerned for this effect (van Giersbergen et al., 2007a).

Distribution, metabolism and excretion of clazosentan were investigated in four healthy male subjects given IV administration of 1 mg/kg ^{14}C-labeled drug. Blood, urine and feces samples were collected for a period of 8 days. Clazosentan was mainly excreted unchanged into feces whereas about 15% of the radioactive dose was recovered in urine. No metabolites representing more than 5% of total radioactivity were identified. No relevant inhibition of the human cytochrome P450 isoenzymes, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4, was observed in vitro at clazosentan concentrations largely exceeding those observed in clinical trials. In human blood, clazosentan was highly bound to plasma proteins and showed no significant penetration into red blood cells. Taken together, these results suggest that Clazosentan is a promising drug for use in clinical trials (van Giersbergen et al., 2009).

In 2005, 34 patients (61% female) with SAH were enrolled to study safety of Clazosentan. 16 received Clazosentan and 18 received placebo control. Overall, the rates, nature, and severity of adverse events were comparable between the two treatment groups. No adverse event pattern indicated a specific organ toxicity of clazosentan. Further the incidence of arterial hypotension was NOT greater than placebo (Vajkoczy et al., 2005). In 2007, these findings were extended to include a separate ethnic comparison (Japanese v. Caucasian). In this study which included 12 Japanese and 12 Caucasian males and females aged 18-50, Headache was the most frequent adverse event, and its incidence was similar in both ethnic groups. Both groups also reported dizziness and feeling hot with similar frequency. In addition to these 3 adverse events, Caucasian subjects incidentally reported other adverse events whereas no other adverse events were reported by Japanese subjects. Three of the 42 reported adverse events were of moderate intensity (all in Caucasian subjects), whereas all others were rated as mild by the investigator. The type of adverse events reported as similar between male and female subjects within each ethnic group, but females reported more adverse events than males (25 vs 17, respectively). Most subjects reporting headache were administered acetaminophen for pain relief. In addition to receiving acetaminophen to treat a headache, 1 Caucasian male subject was administered a single 10-mg dose of metoclopramide to treat nausea. All adverse events resolved without sequelae (van Giersbergen et al., 2007b).

C. Preliminary Data

In Specific AIM 1 we will determine a dose of Clazosentan that blocks the observed hypoperfusion following TBI. Figure 1 shows preliminary data in which 1 mg/kg Clazosentan was administered IV at 2 h post injury. This dose appears to be effective in blocking the hypoperfusion.

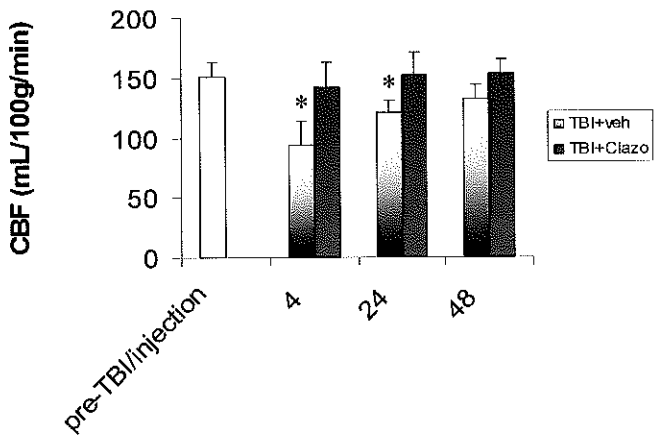


Figure 2. CBF was measured in 8 rats prior to injury (pre-TBI/injection, white) using ASL-MRI. Either veh (black and white) or 1 mg/kg Clazosentan (blue) was given IV at 2 h post TBI. While whole brain scans were acquired, area of analysis in the data presented, here, was limited to the hippocampus due to its strong association with learning. Preliminary results (N=4 per group) show that Clazosentan is able to ameliorate TBI-induced hypoperfusion. *p<0.05 as compared with pre-TBI (analyzed using ANOVA with LSD post hoc).

We will also aim to determine a dose which is effective in improving behavioral deficits following TBI. Therefore we also tested performance on a radial arm maze following an injection of 1 mg/kg Clazosentan at 2 h post injury. While conducted in only a limited number of animals (N=6 for Clazo, N=8 for Sham and N =10 for TBI), results suggest that 1 mg/kg Clazosentan leads to a partial improvement in behavioral outcome (i.e., rats did not perform as poorly as TBI animals, however were not as successful as sham operated animals). Therefore, further dosing and dosing regime will be required to optimize outcome.

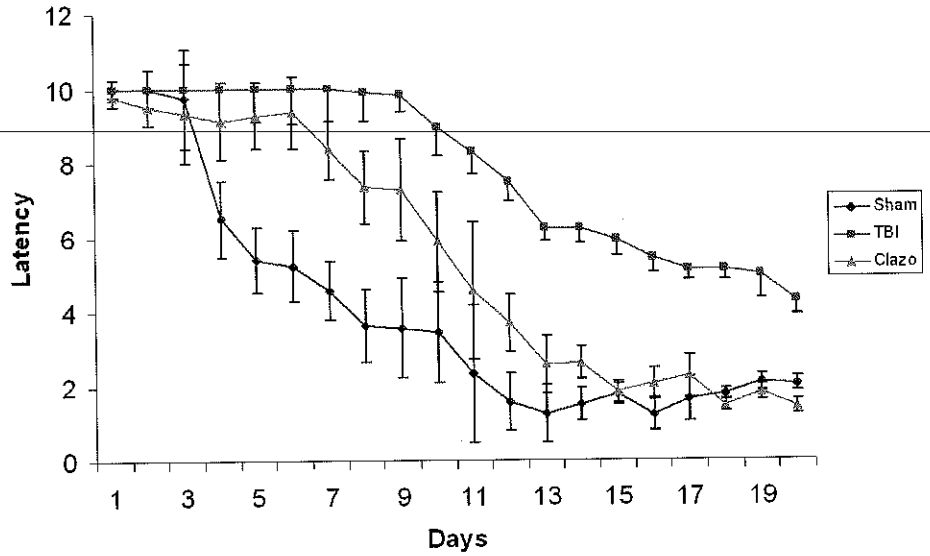


Figure 3. 1 mg/kg Clazosentan was administered 2 h post TBI (N=6). Latency (the time it takes to retrieve 4 food incentives) was measured for 21 days to test performance in a radial arm maze. These results were compared with those obtained from sham operated (N=8) and TBI animals (N=10). Results indicate that this dose of Clazosentan given at 2 h post TBI leads to a partial improvement in behavioral outcome.

In Specific AIM 2 we want to recapitulate results gained from rat in a porcine model of TBI. In order to show that the porcine model mimics the rat model with regards to changes in CBF after TBI, we have included Figure 4 which shows that CBF, as in rats, is significantly reduced in pigs after TBI.

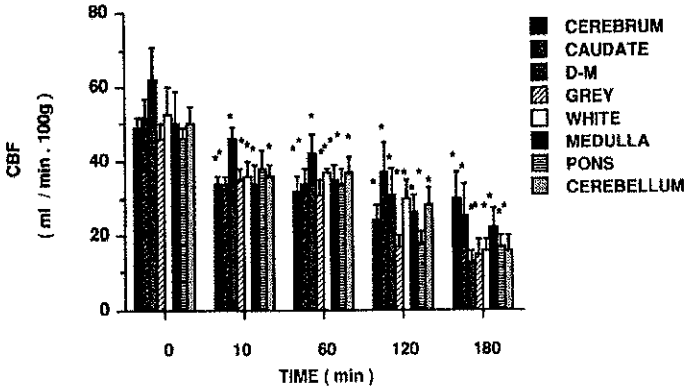


Figure 4. Influence of FPI (2 atm) on regional CBF in pigs, n=5. *p<0.05 compared to corresponding 0 time point (pre-injury) in multiple areas of the brain. Please note that, as in the rat, while we have the ability to measure multiple brain areas, in this proposal we will focus on hipp and smCx due to their known functional association with memory and learning.

Further, in order to show proof of concept that ETrA antagonism improves blood flow after TBI, Figure 5 shows the results of injection of BQ-123 in pigs at various time points following TBI. Results indicate that ETrA antagonism improves CBF following TBI in pigs.

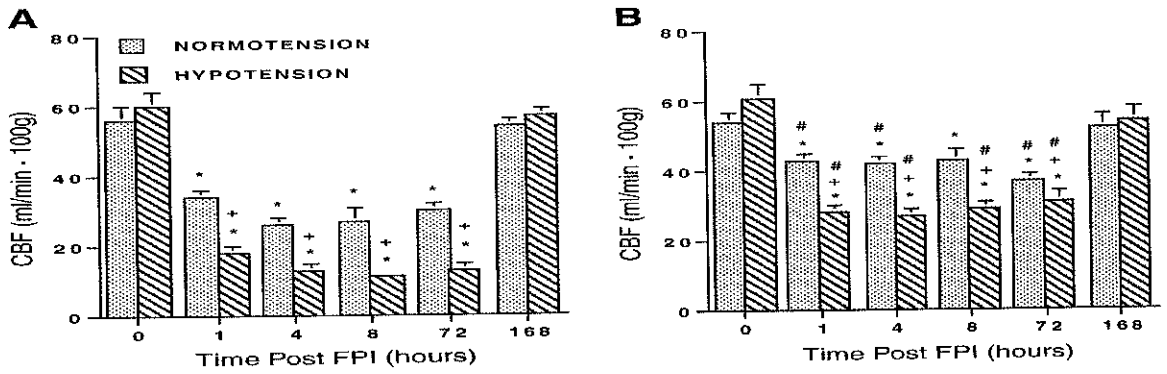
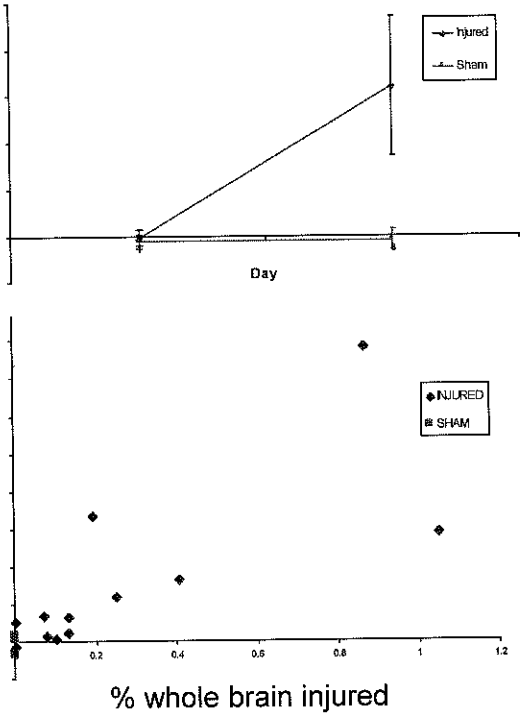


Figure 5. Influence of FPI (2 atm) on total CBF during normotension and hypotension (45% decrease in MAP acutely for 10 min) in absence (Panel A) and presence of BQ-123 (1 mg/kg iv) (Panel B) in pigs, n=5. *p<0.05 compared to 0 time point (pre-injury) +p<0.05 compared to corresponding normotension value #p<0.05 compared to corresponding non BQ-123 treated value (Panel A).

Also key to this proposal is the ability to measure behavioral outcome in pigs. To describe the overall neurobehavioral performance of pigs and correlate it with severity of neuropathology, we developed a composite cognitive dysfunction score. Neurobehavioral outcome measures exhibited a wide range of variability within each injury group (coefficient of variance 15%-220%). The basis for the composite score was a set of neurobehavioral tests with the most consistent responses among the SHAMs (coefficient of variance ≤ 40%). Five neurobehavioral measures were included: T-maze training failure rate, T-maze intra-maze change time in contact with novel object, latency to food reward for T-maze normal trials and T-maze reversal trials, and sniffing the walls from open field testing which include measurements of executive function, memory, learning, reverse learning, and problem solving.

Figure 6



Scores for each of the five behavior measures were tallied to calculate a cognitive composite dysfunction score. Composite cognitive dysfunction scores did not differ between the groups on Day 1, but on Day 8, INJ scores were significantly higher, with no change in SHAMS (Figure 6, top). Composite cognitive dysfunction scores from Day 8 correlated well with percent white matter injured as assessed by β-APP immunoreactivity, with a correlation coefficient of 0.76 (Figure 6, bottom). *These results demonstrate the feasibility of our cognitive behavioral performance assessments and that we have a well-characterized porcine neurofunctional assessment tool that has been validated against histological measures of brain injury volume.*

D. Approach: Research Design and Methods

SPECIFIC AIM 1. Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either 2 or 8 h post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a rodent model of TBI.

Goals and timeline for *YEAR 1*: Determine whether candidate pharmacotherapy has characteristics which warrant proceeding to testing in a porcine model. ***(10months)***

Rationale for *Year 1*: Published and preliminary data from our laboratories show that endothelin-1, a powerful vasoconstrictor, is upregulated following TBI. Further, we have shown that one of its receptors, ETrA, which mediates vasoconstriction, is upregulated as early as 4 h post injury. This finding temporally corresponds with observed vasoconstriction and hypoperfusion. Data from several laboratories shows

that BQ-123, a non-clinically relevant ETrA antagonist, blocks TBI induced vasoconstriction. A recent publication from Dr. Kreipke's laboratory shows that blockade of ETrA using BQ-123, ameliorates hypoperfusion and reduces the extent of cell injury (Kreipke et al., 2009). Furthermore, preliminary data shows that BQ-123 improves behavioral outcome (Kreipke et al., 2009). With the recent production of a clinically relevant ETrA antagonist, and with the mutual agreement between Actelion and Dr. Kreipke to use this drug, we have generated preliminary data for this U01 application which shows that an IV injection of Clazosentan given 2 h following TBI recapitulates the results of BQ-123. Therefore, if funded, we will extend these findings to determine the optimal dose of Clazosentan needed and the optimal range of times (2 or 8 h post injury) that can be given to improve outcome.

Experimental Approach for *Year 1*:

Single bolus, 2 h:

In order to establish baseline, 24 h prior to TBI, all rats (90 animals) will have an initial ASL scan to determine CBF (CBF measurements will be taken as described in General Methods) in sensorimotor cortex (smCx) and hippocampus (hipp) due to the association of these anatomical areas with learning and memory. Animals (15 per group, groups = sham, TBI only, TBI+veh, TBI+0.1mg/kg, TBI+1.0mg/kg, TBI+10mg/kg Clazosentan) will be either sham operated or given TBI using the weight drop method described in General Methods. Following surgery, animals will be allowed to recover for 2 hours at which time they will receive no injection (TBI only), veh, or one IV injection of the selected dose of Clazosentan. Dosing range is based on established protocols in animals (Vater et al., 2005a,b) and on tolerability and safety data in humans involved in the SAH clinical trial (Vajkoczy et al., 2005; van Giersbergen et al., 2007a,b; 2009). At 4 h post injury a subset of 6 animals per group will be randomly selected for ASL CBF measurements. Animals will then be returned to home facility. At 24 h post injury, CBF measurements will be retaken in the same subset of 6 animals per group. Four h post CBF measurement (to allow for full recovery from anesthesia) all animals (15 per group) will be tested for motoric performance. As described below, any animal not performing at normal, non-treated levels will be discarded as this will confound interpretation of behavioral data. At 48 h post injury, CBF will be measured once again in the same subset of 6 animals per group as before. Four h after CBF measurements, behavioral data acquisition will commence. All animals (15 per group) will be tested for cognitive performance using the radial arm maze as described below in General Methods for 20 days (testing done from day 2 through day 20). Comparison across groups will be conducted using ANOVA with LSD post hoc to determine whether Clazosentan has an effect on either CBF or cognitive behavior following TBI.

-24h 0h 2h 4h 24h 28h 48h 52h 22d
ASL CBF → TBI/sham → injection → ASL CBF → ASL CBF → motoric testing → ASL CBF → behavioral testing → end

Single bolus, 8 h:

In order to establish baseline, 24 h prior to TBI, all rats (90 animals) will have an initial ASL scan to determine CBF in smCx and hipp due to the association of these anatomical areas with learning and memory. Animals (15 per group, groups = sham, TBI only, TBI+veh, TBI+0.1mg/kg, TBI+1.0mg/kg, TBI+10mg/kg Clazosentan) will be either sham operated or given TBI using the weight drop method described in General Methods. Following surgery, animals will be allowed to recover for 8 hours at which time they will receive no injection (TBI only), veh, or one IV injection of the selected dose of Clazosentan. Two hours post injection, CBF measurements will be taken in a subset of 6 randomly chosen animals per group. At 24 h post injury, CBF measurements will be retaken. Four h post CBF measurement (to allow for full recovery from anesthesia) all animals (15 per group) will be tested for motoric performance. As described below, any animal not performing at normal, non-treated levels will be discarded as this will confound interpretation of behavioral data. At 48 h post injury, CBF will be measured once again in the same subset of 6 animals per group as before. Four h after CBF measurements, behavioral data acquisition will commence. All animals (15 per group) will be tested for cognitive performance using the radial arm maze as described below in General Methods for 20 days.

-24h 0h 8h 10h 24h 28h 48h 52h 22d
ASL CBF→ TBI/sham → injection → ASL CBF→ASL CBF → motoric testing → ASL CBF → behavioral testing → end

Two injection approach, 2 and 24 h:

Same as single bolus approach, however an additional IV injection of Clazosentan will be given at 24 h. Second injection will match the dose of the first injection (e.g., 1.0 mg/kg given at 2 h and 24 h).

-24h ASL CBF → 0h TBI/sham → 2h 1st injection → 4h ASL CBF → 24h 2nd injection → 25h ASL CBF → 28h motoric testing → 48h ASL CBF → 52h behavioral testing → 22d end

Two injection approach, 8 and 24 h:

Same as single bolus approach, however an additional IV injection of Clazosentan will be given at 24 h. Second injection will match the dose of the first injection (e.g., 1.0 mg/kg given at 8 h and 24 h).

-24h ASL CBF → 0h TBI/sham → 8h 1st injection → 10h ASL CBF → 24h 2nd injection → 25h ASL CBF → 28h motoric testing → 48h ASL CBF → 52h behavioral testing → 22d end

Milestone for *Year 1*: A particular dose and dosing regime of Clazosentan will reduce the extent and duration of hypoperfusion and will improve behavioral outcome after TBI. *If* determined, *then* we will proceed to Specific Aim 2, Year 2.

Criteria for success for *Year 1*: TBI results in an approximately 30-35% reduction in blood flow followed by vasospasm in rats. This observation is recapitulated in humans. Therefore, we will quantitate CBF using ASL-MRI, a technique routinely used in both small animal models as well as humans, following drug administration. Improvement will be defined as an increase in CBF of 20% or greater, thus reducing hypoperfusion to 15-20% following TBI. Success will be defined by 70% of the animals per treatment group achieving this improvement. Furthermore, published data from our laboratory shows that TBI extends the time needed to learn behavioral tasks by minimally 7 days and increases number of errors by 20%, which when combined to produce a cognitive score (cs) (see statistical methods for derivation of equation) results in a reduction in cs by >40% (or <60% of control). Therefore, in the same animals in which CBF is determined, we will test behavioral outcome following drug treatment. Improvement in behavioral testing will be defined as an increase in cs to >60% of control. Success will be defined by 70% of the animals per treatment group reaching this improvement goal.

SPECIFIC AIM 2. Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either 2 or 8 h post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a porcine model of TBI.

Goals and timeline for *YEAR 2*: Determine whether candidate pharmacotherapy given at 2 h post injury has characteristics (i.e., improves CBF and behavioral outcome) that would warrant testing a later injection time (8 h). ***(12 months)***

Rationale for *YEAR 2*: Many TBI studies use rodent models. However, rodents have a paucity of white matter. Pigs provide many advantages in modeling the human brain. The overall shape, gyral pattern, and distribution of gray and white matter are similar in pigs and humans. The growth pattern of the postnatal brain is similar to that of human infants. The response of the pig to hypoxia and ischemia parallels that observed in humans. CBF, metabolism, and maturation of pigs is similar to the human. Selective white matter vulnerability in humans similarly occurs in pigs with acute subdural hematoma. The gyrencephalic pig brain containing substantial white matter is appropriate to model human TBI. Due to these potential advantages in choosing a porcine model, we will aim to recapitulate the data gained in Year 1 in rats. Therefore, we will measure CBF at the same time points, in the same regions, using the same technique (ASL-MRI) as outlined in the studies in rats in order to test the efficacy of Clazosentan given at 2 or 8 h post injury in two distinct animal models of diffuse brain injury. Further, we will test behavior in the same animals, using a similar apparatus as that used in rat but designed for the pig.

Experimental Approach for *Year 2*:

If, as our preliminary data suggests, we are able to determine an effective dose and "window of opportunity" for Clazosentan in the rat model of TBI, we will then extend these results to include data from a more human-like model, the pig. The dosing range that will be studied in the pig studies will be derived from the information that we determined in the rat. Thus, the rat studies will inform the design of the pig studies. In Table 1, for example, D1 will be the dose found to be effective in the rat, while D2 may be 3 X D1, in order to gain further dose-response information in the pig. In year 2, animals will receive fluid

percussion brain injury and will be treated either at 2 h, 2 h and 8 h, or 2 h, 8h and 24 h (ie. single bolus or multi-injection), and two doses will be examined (D1, D2) along with vehicle treated animals (Table 1, below). Studies will also be undertaken on sham operated animal receiving the same doses. Cerebral blood flow will be measured with ASL MRI the day prior to fluid percussion brain injury, 2 h following the first treatment (6 h after injury) as well as 48 h after injury (Table 1, below). Behavior will be evaluated at 72 h and 7 d.

Group #	Year	Treatmt	Injury	Time Rx1	Time Rx2	Time Rx3	CBF	Behav
1	2	D1	Y	2	--	--	-1D, 6H, 48H	72H,7D
2	2	D1	Y	2	8	--	-1D, 6H, 48H	72H,7D
3	2	D1	Y	2	8	24	-1D, 6H, 48H	72H,7D
4	2	D2	Y	2	--	--	-1D, 6H, 48H	72H,7D
5	2	D2	Y	2	8	--	-1D, 6H, 48H	72H,7D
6	2	D2	Y	2	8	24	-1D, 6H, 48H	72H,7D
7	2	V	Y	2	8	24	-1D, 6H, 48H	72H,7D
8	2	V	N	2	8	24	-1D, 6H, 48H	72H,7D
9	3	D1	Y	--	8	--	-1D, 8H, 48H	72H,7D
10	3	D1	Y	--	8	24	-1D, 8H, 48H	72H,7D
11	3	D2	Y	--	8	--	-1D, 8H, 48H	72H,7D
12	3	D2	Y	--	8	24	-1D, 8H, 48H	72H,7D

D1 = dose 1; D2 = dose 2; V = vehicle; Y=yes; N=no; treatment times in hours; H=hours; D=days
Time Rx1, Rx2, Rx3 are time in hours following injury.

Milestone for *Year 2*: A particular dose and dosing regime of Clazosentan given at 2 h post TBI will reduce the extent of hypoperfusion and will improve behavioral outcome following injury. *If* determined, *then* we will proceed to injection of Clazosentan at 8 h post TBI in Year 3.

Criteria for success for *Year 2*: Using optimal dose and dosing regime as determined in rat in Year 1, improvement will be defined as a reduction in CBF to only 15-20% of baseline. Success will be defined by 70% of the pigs per group achieving the improvement goal. Behavioral deficits in pigs is defined as a composite cognitive dysfunction score (ccds). Poor outcome is defined as a ccds > 3. Therefore improvement will be defined by a reduction in the score below 3. Success will be defined by 50% of the animals per group improving below a 3 ccds when drug is administered at 2 h post injury.

Goals and timeline for ***YEAR 3***: Determine whether candidate pharmacotherapy given at 8 h post injury has characteristics (i.e., improves CBF and behavioral outcome) that would warrant a successful application for an IND ***(12 months)***

Rationale for *Year 3*: By testing administration of Clazosentan at a longer time point post TBI (8 h) we will identify a diverse range of drug delivery times which will improve outcome.

Experimental Approach for *Year 3*:

In year 3, the first dose will be administered at 8 h to examine the effect of delayed treatment with some groups treated also at 24 h (Table 1). In these animals CBF will be measured 2 h post first treatment (10 h post injury) and at 48 h. Behavior will be examined at the same times as outlined above for year 2 animals.

Milestone for *Year 3*: A particular dose and dosing regime of Clazosentan given at 8 h post TBI will reduce the extent of hypoperfusion and will improve behavioral outcome following injury. *If* determined, *then* we will proceed to Year 4 Terminal Milestone.

Criteria for success for *Year 3*: Using optimal dose and dosing regime as determined in rat in Year 1, success will be defined as in Year 2.

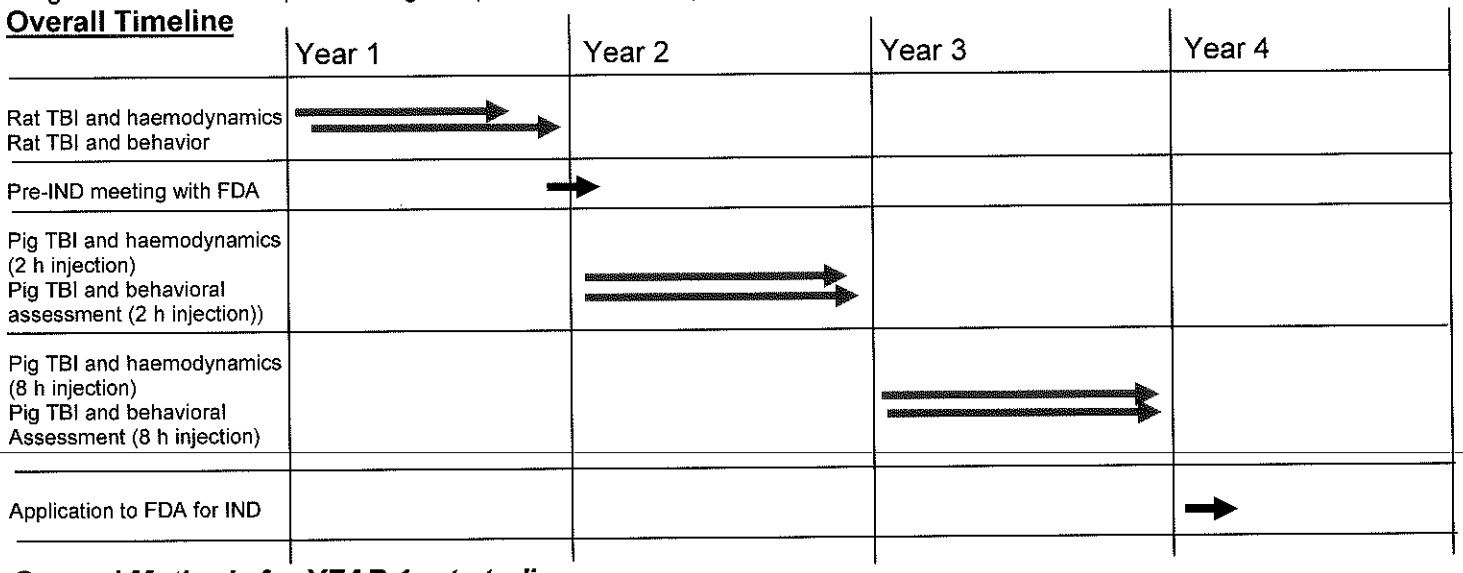
Terminal Milestone*: *Year 4 application for IND status with FDA

Goals and timeline for *Year 4*: Prepare application for IND ***(3 months)***

Criteria for success for *Year 4*: Successfully obtain IND status to move forward with clinical trial.

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian, W.

Overall Timeline



General Methods for YEAR 1 rat studies:

Closed head trauma model in rat:

Male Sprague-Dawley rats (Sprague-Dawley) will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing. Halothane will be used as the anesthetic for all experiments. The use of halothane instead of the more recently introduced isofluorane is preferred because of recent evidence indicating the latter neuroprotective properties ([Zhao et al., 2007; Wei et al., 2007] and more recent data presented at the Brain '07, International Cerebral Blood Flow and Metabolism meeting held in Osaka, Japan). A midsagittal scalp incision will be performed and the underlying muscles retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skull over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, thus preventing skull fractures with penetrating brain injury. After the cement is allowed to dry for three minutes, the animals will be placed prone on a platform as described in the acceleration impact trauma model of Marmarou (Marmarou et al., 1994). After 30-40 seconds of placement, 450g of weight contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 meters. Following a brief convulsion and respiratory arrest, most animals restart breathing on their own. However, in some cases, the use of a rodent respirator or CPR is necessary prior to spontaneous breathing. Using these precautions, in our hands mortality has been reduced to less than 5%. In some animals after impact, the helmet will be removed and the skin sutured only if the skull shows no evidence of fractures. After suturing the skin, sensory cutaneous and evoked motor responses will be tested. Usually the intubation tube is removed at 10 minutes post trauma and only animals which are able to right themselves before 30 minutes after injury will be included in the study (Petrov et al., 2000; Petrov et al., 2002). Brain and leg muscle temperatures will be taken routinely, in some instances up to 24 hrs post injury. We have determined that brain temperature fluctuated only 1.5ºC, and muscle temperature 1.3ºC, during this time period.

CBF measurements:

CBF measurements will be acquired using a standard protocol which is routinely used by our laboratory (Shen et al., 2007; Kreipke et al., 2009). Briefly, prior to image acquisition, anesthesia will be induced by a steady application of 1% halothane using a specially designed apparatus compatible with the MRI to sedate the animals. The animal will be placed in a prone position on a cradle with a custom-built palate holder equipped with an adjustable nose cone and stereotaxic ear bars in order to minimize movement during MRI scans.

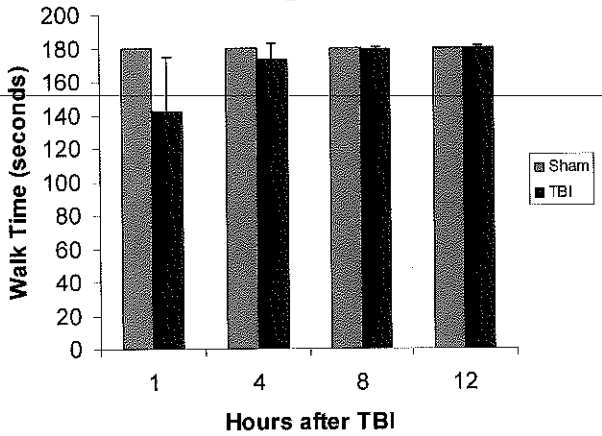
The rat head will be positioned at the isocenter of a magnet. MRI scans will be repeated at four time points. Baseline scans will be run before TBI is induced, and then as indicated in the experimental design. All MRI measurements will be performed on a 4.7-T horizontal-bore magnetic resonance spectrometer (Bruker AVANCE) with an 11.6-cm-bore actively shielded gradient coil set capable of producing a magnetic field gradient of up to 250 mT/m. A whole-body birdcage radiofrequency (RF) coil (inner diameter, 72 mm) will be used as the transmitter for homogeneous RF excitation, and a surface coil (30

mm diameter) will be used as the receiver, with active RF decoupling to avoid signal interference. Two sequences will be run: T1 weighted image (used to identify brain regions and permit alignment of the two ASL-CBF scans that will be obtained in each animal) and ASL will be run for the measurement of flow. For all sequences, the field of view will be 40X40X24 mm^3; thus, the whole brain will be imaged. While we would, therefore, be able to measure any anatomical area in the brain, we will focus on smCx and Hipp due to their functional relationship to learning and memory. The remaining imaging parameters used are as follows: ASL: TR = 1550 ms, TE = 7.65 ms, matrix size NxXNy =128X70 (interpolated by zero filling in k-space to 256_256), slice=1 (thickness, 2 mm), Nacq=2, labeling slice=2 cm offset from isocenter, adiabatic fast passage with Magnetization transfer contrast (MTC) gradients=1.5 s, spin echo=3;

Assessment of motor deficit:

Since neurological deficits, while rarely seen using this model of TBI, would greatly hinder the ability of a rat to perform on the radial arm maze or in a Morris Water Maze, all animals will be screened following TBI for neurological outcome. In order to screen animals for motor deficit, all TBI animals will be tested using standard neurological function tests, including rotor rod performance, balance beam, and ladder climbing. Based on preliminary screens (Figure 7), rats either performed well or, on the contrary, showed deficit on all tests and, therefore, animals performing at sub-control levels on any test will be grounds for removal from the study.

Figure 7



Behavioral testing and radial arm maze setup:

Behavioral testing will be conducted using a previously published protocol in our laboratory (Kreipke et al., 2008). Briefly, rats will be allowed to acclimate to their new environment (in DLAR facility) after their arrival. Then from day 1 to day 3 of the behavioral study the rats will be handled by the researcher for 10 to 15 minutes each. Acclimation to the maze environment also will be initiated during which the rats will be placed on the central platform of the radial arm maze and allowed to roam freely.

A custom designed radial arm maze will be built using black acrylic sheet (0.6 cm thick). Eight identical radial arms are fixed to an octagonal base platform that stands 63 cm above the floor. Each radial arm is 60 cm in length and 10 cm in width with 10 cm – high sidewalls along each arm. At the end of each arm a 5-cm end piece is placed. A hole measuring 2.5 cm in diameter is also cut 5 cm from the end of each radial arm to place a plastic food cup (1 oz). During behavioral testing, the maze is enclosed within four black linen walls. A white paper triangle (15-cm sides) is placed on one linen wall 10 cm above the base of radial arm #3. An 8" x 11" white paper square with bisecting black lines is placed on the same linen wall 10 cm above the base of radial arm #5. These visual cues are aimed to provide spatial guidance as to the location of the baited arm (i.e. containing the food).

The rats will be tested for the time taken to find the bait (half of a Fruit Loop cereal®) placed in a plastic cup of four different radial arms. Also the number of spatial learning (entering an unbaited arm) and memory retention (re-entering a baited arm after the food has been removed) errors will be recorded. All data is automatically obtained using an overhead camera linked to Smart™ Version 2.5 software (San Diego Instruments, San Diego, CA) Each rat will be tested daily for two consecutive time trials over a period of 20 days. The maximum time a rat will be allowed to spend in the maze is ten minutes per trial by the end of which is determined to be conclusion of a trial. Averages of these trials will be calculated and recorded.

General Methods for Years 2 and 3 porcine studies:

Closed head trauma model in pig.

Methods for brain FPI have been described previously (Armstead 1996). A device designed by the Medical College of Virginia is used. A small opening is made in the parietal skull. A metal shaft is sealed into the opening on top of intact dura. This shaft is connected to the transducer housing, which is in turn connected to the fluid percussion device. The device itself consists of an acrylic plastic cylindrical reservoir 60 cm long, 4.5 cm in diameter, and 0.5 cm thick. One end of the device is connected to the transducer housing, whereas the other end has an acrylic plastic piston mounted on O-rings. The

exposed end of the piston is covered with a rubber pad. The entire system is filled with 0.9 % saline. The percussion device is supported by two brackets mounted on a platform. FPI is induced by striking the piston with a 4.8 kg pendulum. The intensity of the injury (1.9-2.1 atm. with a constant duration of 19-23 ms) is controlled by varying the height which the pendulum is allowed to fall. The pressure pulse of the injury is recorded on a storage oscilloscope triggered photoelectrically by the fall of the pendulum. The amplitude of the pressure pulse is used to determine the intensity of the injury.

Survival surgery of brain injured pigs.

Male pigs will be anesthetized with halothane (2-3 MAC) and aseptic technique used to surgically place the adaptor for connection to the brain injury device. After injury, the animal will be allowed to recover and experimental protocols followed for behavioral assessment and determination of cerebral blood flow by ASL MRI. Six hours after FPI the pig will be anesthetized with halothane and taken to the MRI facility for measurement of blood flow (see descript of CBF Measurements below). After the imaging session, the animal will be returned to his cage for recovery, and kept warm ($\approx 37^{\circ}$ C) until awake. This procedure will be repeated 48 hours after FPI.

CBF measurements.

Cerebral blood flow will be measured using a pseudo-continuous arterial spin label (pCASL) technique routinely used in the Center for Functional Neuroimaging. For all three sessions (prior to FPI and 6 (or 8) and 48 h post FPI), the pig will be anesthetized with halothane and placed into the MRI scanner (Siemens TIM Trio) and a volume transmit/8-channel receive knee coil positioned on the head. The head will be held steady using a MR-compatible stereotaxic headholder. This positioning device will permit the head to be positioned similarly for both imaging sessions. The following acquisition parameters will be used: gradient-echo echoplanar imaging with TR/TE = 4000/17 ms, FOV= 25 cm, matrix = 64 x 64, slice thickness = 4 mm (yielding 4 mm isotropic resolution), flip angle = 90°, labeling time = 2 sec with a post-label delay of 1.2 sec, 120 ta/control pairs (16 min). Pior to the pCASL measurement, a structural MR image will be acquired with 1 mm isotropic resolution using a T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence. This image will be used to identify brain regions and permit alignment of the two ASL-CBF scans that will be obtained in each animal.

Behavioral measurements.

Our previously published neurofunctional assessments (open field and T-maze) (Freiss et al, 2007) will be performed on 3 days and 7 days post-injury to evaluate memory, learning and executive function. In addition to all injured treatment groups, uninjured sham animals will receive all behavior testing. These shams are used as the basis for the composite cognitive dysfunction (CCD) z-score calculations, described below. The animals will be fasted for at least 6 hours before the first test begins. Testing order will be randomly assigned, and all tests will be on DVD for scoring by a blinded evaluator. All procedures involve operant conditioning techniques with food as a positive reward. Each animal will be placed in an open field (4 x 8 ft) with various objects placed in predetermined locations. The behaviors (e.g. nudging toys, walking, sleeping) will be scored as present/absent for every minute-long interval (Martin, P. and P. Bateson, 1986). The test provides a measure of exploratory interest which involves a high level of sensory processing (Kelly et al., 2001), and an intact prefrontal cortical-striatal-pallidal circuit and concomitant cerebellar function (Pierce, K. and E. Courchesne, 2001). Cognitively impaired individuals are inactive for longer periods and interact with and explore objects less (Head et al., 1997) The T-maze test will assess memory and cognition by requiring that they recall the location of a food reward (Bolhuis et al., 2004). This task is profoundly affected in cognitively impaired individuals (Head et al., 1995). After successful training, a novel object will be placed in the food reward arm of the T-maze, and time in contact with the novel object and latency to food reward will be recorded. We find increased latency to food reward by injured animals compared to instrumented sham animals. Finally, the food reward will be switched as a test of executive function and reversal learning (Adams et al., 2000). Time needed to complete the maze and number of times incorrect choices made will be scored.

Neurobehavioral outcome measures in pigs can exhibit a wide range of variability within each animal group. To improve the description of the overall neurobehavioral performance of pigs, we have developed a CCD score to evaluate overall neurofunction relative to the sham animals (Friess et al., 2009). The basis for the composite score is a set of neurobehavioral tests with the most consistent responses among previous SHAM groups (coefficient of variance ≤ 40%). Five neurobehavioral measures are included: T-maze training pass rate, T-maze intra-maze change time in contact with novel object, latency to food

reward for T-maze normal trials and T-maze reversal trials, and sniffing the walls from open field testing. Together, these outcome measures assess executive function, memory, learning, reverse learning, and problem solving. First, for each of the five measurements, mean and standard deviation for SHAM are calculated for each behavior. Next, each injured animals' performance on the selected behaviors is compared to the SHAM mean and standard deviation. The injured animal's score is calculated for each behavior by taking the differences between the individual animal's performance and the SHAM mean. The difference is then divided by the standard deviation of the SHAM group to obtain a z-score for that pig for that assessment. Negative scores are used for individual performances that are below the SHAM mean for T-maze intra-maze change, T-maze normal trials, and T-maze reversal trials. Negative scores are also used for individual performances that are above the SHAM mean for T-maze pass rate and sniffing the walls in open field testing. Scores for each of the five behavior measures for an individual subject are summed to calculate the composite CCD score for that animal.

Statistical Analyses

Note: all statistical analyses will be conducted in accordance with Wayne State University's Biomedical Statistical Core facilities guidelines. Wayne State University's Core facility offers free consultation with a number of their staff at any time during the course of a funded project.

CBF measurements. All data pertaining to CBF are expressed as the average of scans taken independently. CBF is expressed as mL/100g/min. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (Shen et al., 2007), in rats we were able to detect significant changes in CBF between groups using 6 animals per group with 95% power at $\alpha = 0.05$. The cerebral blood flow data in the pig will be analyzed in a similar fashion to that in the rat. CBF will be obtained by pCASL prior to the fluid percussion injury, as well as acutely (6 or 8 hrs depending upon treatment time) and chronically (48 hrs) post injury. The ASL-MRI technique provides quantitative data of CBF with 4 mm resolution in the main structures of interest (smCx and Hipp) as well as in most of the remainder of the brain since the axial field of view will be 48 mm. This will permit an ANOVA analysis with blood flow in each region post injury compared to that prior to injury. With expected variability of the blood flow data, we expect to be able to detect significant changes in CBF with 10 animals/group with a power of 80% and α=0.05, similar to prior studies.

Behavioral Assessments. All behavioral data in rats are expressed as the average over two trials per animal of an overall cognition score (cs) that incorporates latency (time it takes to retrieve all four Fruitloops™), spatial learning errors (error in which animal goes down an unbaited arm), or memory retention errors (error in which animal reenters a baited arm after food is already removed). The cs is determined by the following equation: cs=1/{(0.77L)X[(0.063E1)+(0.031E2)+1]}, where L=latency in min, E1=spatial learning error, and E2=memory retention error. This equation was developed to incorporate both errors and latency into an overall assessment for cognition. This equation is based on trials with normal animals (no treatment) in which the fastest time on the maze in 1 min 18 seconds with no errors. These measures were designed, in part, to allow for an overall measure of performance and comparison of data across behavioral paradigms, across species. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. In rats, due to the variability in behavior amongst individual animals, we have previously determined (Kreipke 2004, 2007) that we can distinguish significant differences between TBI and control animals using 12 animals per group. Antagonist studies will require 12-15 animals per group to show an improved performance with power of 90%. This allows us to distinguish a difference in cs of 10 with 90% power at $\alpha = 0.05$. Additional rats may be required due to failure to exercise, death, or motoric disability. In pigs, behavioral data are expressed as a composite cognitive dysfunction score (ccds) as described above in the behavioral measurements section. With success defined for each animal as ccds<3, we will determine the average success rate for each group. Success greater than 50% will be required for a dosing regime to be considered for translation to clinical trials. All calculations for ccds assume a one-sided Type I error level of$\pm$=0.05 with 80% power. The preliminary data presented for pigs with diffuse white matter damage demonstrated a ccds of approximately 15.9 ± 22.6 in injured animals on Day 8. Thus, an effect size of 0.65 times the standard deviation corresponds to a difference of 14.7 points. The corresponding within-group (two-sided) 95% confidence intervals for a mean will be ±0.62 times the standard deviation for n=10.

Adams, B., et al., (2000).The canine as a model of human cognitive aging: recent developments. Prog Neuropsychopharmacol Biol Psychiatry, 24: p. 675-92.

Andersson KE. Neurophysiology/pharmacology of erection. (2001) Int J Impot Res. 13 Suppl 3:S8-S17.

Armstead WM. (1996) Role of endothelin in pial artery vasoconstriction and altered responses to vasopressin after brain injury. J Neurosurg. 85(5):901-907.

Armstead WM. (1999) Superoxide generation links protein kinase C activation to impaired ATP-sensitive K+ channel function after brain injury. Stroke. 30(1):153-9.

Armstead WM, Cines DB, Al-Roof Higazi A. (2004) Altered NO function contribures to impairment of uPA and tPA cerebrovasodialation after brain injury. J Neurotrauma 21:1204-1211.

Barone FC, Ohlstein EH, Hunter AJ, Campbell CA, Hadingham SH, Parsons AA, Yang Y, Shohami E. (2000) Selective antagonism of endothelin-A-receptors imporves outcome in both head trauma and focal stroke in rat. J Cardiovasc Pharmacol. 36 (Suppl 1):S357-S361.

Battistini B, Berthiaume N, Kelland NF, Webb DJ, Kohan DE. (2006) Profile of past and current clinical trials involving endothelin receptor antagonists: the novel "-sentan" class of drug. Exp Biol Med (Maywood). 231(6):653-95.

Benigni A, Remuzzi G. (1999) Endothelin antagonists. Lancet. 353(9147):133-8.

Benigni, A., (ed), (2007). Proceedings of ET-10. Bergamo, Italy.

Bolhuis, J.E., et al.(2004) Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. Behav Brain Res,. 152(2): p. 351-60.

Dawson DA, Sugano H, McCarron RM, Hallenbeck JM, Spatz M. (1999) Endothelin receptor antagonist preserves microvascular perfusion and reduces ischemic brain damage following permanent focal ischemia. Neurochem Res. 24(12):1499-505.

Gorlach C, Hortobagyi T, Hortobagyi S, Benyo Z, Wahl, M. (2001) Inhibition of endothelin-1 by the competitive ETA receptor antagonist Ro 61-1790 reduces lesion volume after cold injury in the rat. Pflugers Arch. 441:844-849

Friess, S.H., et al., (2007). Neurobehavioral functional deficits following closed head injury in the neonatal pig. Exp Neurol, 204(1): p. 234-43.

Friess, S.H., et al., (2009) Repeated traumatic brain injury affects composite cognitive function in piglets. J Neurotrauma

Head, E., et al., (1995). Spatial learning and memory as a function of age in the dog. Behav Neurosci, 109(5): p. 851-8.

Head, E., et al.,(1997). Open field activity and human interaction as a function of age and breed in dogs. Physiology & Behavior, 62(5): p. 963-71.

Huttemann M, Lee I, Kreipke CW, Petrov T. (2008). Suppression of iNOS prior to traumatic brain injury improves cytochrome oxidase activity and normalizes cellular energy levels. Neuroscience 151:148-151.

Ishikawa K, Fukami T, Nagase T, Fujita K, Hayama T, Niiyama K, Mase T, Ihara M, Yano M. (1992) Cyclic pentapeptide endothelin antagonists with high ETA selectivity. Potency- and solubility – enhancing modifications. J Med Chem. 35:2139-2142.

Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami, Ozaki S, Nagase T, et al. (1994) Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ788. Proc Natl Acad Sci USA. 91:4892-4896.

Jacobs A, Preston IR, Gomberg-Maitland M. (2006) Endothelin receptor antagonism in pulmonary arterial hypertension—a role for selective ET(A) inhibition? Curr Med Res Opin. 22(12):2567-74.

Kelley, A., M. Cador, and L. Stinus, Exploration and its measurement: a pyschopharmacological perspective, in Psychopharmacology, A. Boulton, G. Baker, and A. Greenshaw, Editors, Humana Press: Clifton, NJ. p. 95-144.

Kreipke CW, Morgan R, Kallikuri S, Rafols J. (2007). Behavioral preconditioning enhances angiogenesis and cognitive outcome following traumatic brain injury. Neurological Research 29:388-394.

Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.

Kreipke CW, Rafols JA. (2009). Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.

Krum H, Liew D. (2003) Current status of endothelin blockade for the treatment of cardiovascular and pulmonary vascular disease. Curr Opin Investig Drugs. 4(3):298-302.

Lüscher TF, Wenzel RR. (1995) Mechanisms of acute coronary syndrome. Schweiz Rundsch Med Prax. 84(6):155-64.

Marmarou A, Foda MA, Van Den Brink W, Campbell J, Kita H, Demetriadou K. (1994) A new model of diffuse axonal injury in rats. Part I: Pathophysiology and biomechanics. J. Neurosurg. 80:291-300.

Martin, P. and P. Bateson, Measuring behavior. (1986) Cambridge UK: Cambridge University Press.

Petrov T, Steiner J, Braun B, Rafols JA. (2002) Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. Neuroscience. 115(1):275-83.

Petrov T, Page AB, Owen CR, Rafols JA. (2000) Expression of the inducible nitric oxide synthase in distinct cellular types after traumatic brain injury: an in situ hybridization and immunocytochemical study. Acta Neuropathol (Berl). 100(2):196-204.

Pierce, K. and E. Courchesne, (2001). Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. Biol Psychiatry 49(8): p. 655-64.

Pollock DM, Schneider MP. (2006) Clarifying endothelin type B receptor function. Hypertension. 48(2):211-2.

Povlishock J. (2008) Progress in neuroprotection: From bench to bedside. Proceedings of the 7th World Congress on Brain Injury, Lisbon, Portugal.

Rafols J, Morgan R, Kallikuri S, Kreipke CW. (2007) Extent of nerve cell injury Marmarou's model compared to other brain trauma models. Neurological Research 29:348-355.

Roux S, Breu V, Giller T, Neidhart W, Ramuz H, Coassolo P, Clozel JP, Clozel M. (1997) Ro 61-1790, a new hydrosoluble endothelin antagonist: general pharmacology and effects on experimental cerebral vasospasm. J Pharmacol Exp Ther. 283(3):1110-8.

Sato M, Noble LJ. (1998) Involvement of the endothelin receptor subtype A in neuronal pathogenesis after traumatic brain injury. Brain Res. 809(1):39-49.

Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. (2007) In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.

Touzani O, Galabraith S, Siegl P, McCulloch J. (1997) Endothelin-B receptors in cerebral resistance arterioles and their functional significance after focal cerebral ischemia in cats. J Cereb Blood Flow Metab. 17:1157-1165.

Uhlmann D. (2006) Clazosentan (Actelion). Curr Opin Investig Drugs. 7(3):272-81.

Vajkoczy P, Meyer B, Weidauer S, Raabe A, Thome C, Ringel F, Breu V, Schmiedek P. (2005) Clazosentan (AXV-034343), a selective endothelin A receptor antagonist, in the prevention of cerebral vasospasm following severe aneurysmal subarachnoid hemorrhage: results of a randomized, double-blind, placebo-controlled, multicenter phase IIa study. J Neurosurg. Jul;103(1):9-17.

van Giersbergen PL, Treiber A, Dingemanse J. (2009) In vitro and in vivo pharmacokinetic characteristics of clazosentan, an intravenous endothelin receptor antagonist, in humans. Int J Clin Pharmacol Ther. 47(3):169-77.

van Giersbergen PL, Dingemanse J. (2007) Effect of gender on the tolerability, safety and pharmacokinetics of clazosentan following long-term infusion. Clin Drug Investig.27(11):797-802.

Vatter H, Zimmermann M, Tesanovic V, Raabe A, Schilling L, Seifert V. (2005a) Cerebrovascular characterization of clazosentan, the first nonpeptide endothelin receptor antagonist clinically effective for the treatment of cerebral vasospasm. Part I: inhibitory effect on endothelin(A) receptor-mediated contraction. J Neurosurg. 102(6):1101-7.

Vatter H, Zimmermann M, Tesanovic V, Raabe A, Seifert V, Schilling L. (2005b) Cerebrovascular characterization of clazosentan, the first nonpeptide endothelin receptor antagonist shown to be clinically effective for the treatment of cerebral vasospasm. Part II: effect on endothelin(B) receptor-mediated relaxation. J Neurosurg. Jun;102(6):1108-14.

Vuurmans JL, Boer P, Koomans HA. (2004) Effects of endothelin-1 and endothelin-1-receptor blockade on renal function in humans. Nephrol Dial Transplant. (11):2742-6.

Wei H, Liang G, Yang H. (2007) Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity. Neurosci Lett. 425(1):59-62.

Zhao P, Peng L, Li L, Xu X, Zuo Z. (2007) Isoflurane preconditioning improves long-term neurologic outcome after hypoxic-ischemic brain injury in neonatal rats. Anesthesiology. 107(6):963-70.

Zwienenberg M, Muizelaar JP. Cerebral perfusion and blood flow in neurotrauma. (2001) Neurol Res. 23(2-3):167-74.

V. VERTEBRATE ANIMALS

The NIH-mandated five points regarding vertebrate animals are addressed as following:

RATS

1. **Provide a detailed description of the proposed use of the animals for the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

   For all experiments, Male Sprague-Dawley rats will be used. Please see below for break-down of animal number per experiment.

2. **Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.**

   The choice to use male Sprague-Dawley rats is based on previous work both in our lab and in the labs cited in the research design. Due to careful use of animals for multiple experiments, no more than 396 male rats (includes 10% exclusion) will be used in total (see experimental design for specific numbers per experiment). Further, rats will be used because of their low cost and because of the large body of information that is now known about their basic neuroanatomy, physiology, and behavior. Rats have an extremely high resistance to infection and are small in size which precludes using large amounts of expensive agents. In addition, the Sprague-Dawley strain has been shown to display pathological changes comparable to those encountered in clinical conditions.

3. **Provide information on the veterinary care of the animals involved.**

   Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility in the same buildings as the laboratories (Dr. Kreipke's laboratory and the Department of Animal Laboratory Research Testing Facility). Training in proper care and handling of animals, as provided by the Wayne State University Department of Laboratory Animal Resources, has been successfully completed by the applicant.

4. **Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.**

   After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection) consistent with our previous work and with the Panel on Euthanasia of the American Veterinary Medical Association. It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own, although a drop of approximately 7% in body weight is expected. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments.

5. **Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.**

Upon termination of a given testing period, rats will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

PIGS

1. **Provide a detailed description of the proposed use of the animals for the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

For all experiments, Male pigs will be used. Please see Table 1 in the Research Plan for break-down of animal number per experiment.

2. **Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.**

The choice to use pigs is based on previous work in our lab. Due to careful use of animals for multiple experiments, no more than 120 pigs will be used in total (see Table 1 of Research Plan). Many TBI studies use rodent models. However, rodents have a paucity of white matter. Pigs provide many advantages in modeling the human brain. The overall shape, gyral pattern, and distribution of gray and white matter are similar in pigs and humans. The growth pattern of the postnatal brain is similar to that of humans. The response of the pig to hypoxia and ischemia parallels that observed in humans. CBF, metabolism, and maturation of pigs is similar to the human. Selective white matter vulnerability in humans similarly occurs in pigs with acute subdural hematoma. Therefore, the gyrencephalic pig brain containing substantial white matter is appropriate to model human TBI.

3. **Provide information on the veterinary care of the animals involved.**

Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility. Training in proper care and handling of animals, as provided by the University of Pennsylvania Department of Laboratory Animal Resources, has been successfully completed by the applicant.

4. **Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.**

After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection). It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments.

5. **Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.**

Upon termination of a given testing period, pigs will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

Multiple-PI leadership Plan and Responsibilities:

This proposal draws on the expertise of both Drs. Kreipke and Armstead in order to gain the most appropriate data required for initiation of IND status with FDA. Both Drs. Kreipke and Armstead are experts in TBI research as it relates to endothelin-mediated hypoperfusion. Their individualized expertise lies in the animal models used to mimic head trauma. Dr. Kreipke is an expert in the rat acceleration-impact model while Dr. Armstead is an expert in the porcine lateral fluid percussion model. Incorporating both cross-model and cross-species data will enhance the application to FDA.

The first year of this application will be conducted in rat only. Therefore, most of the responsibilities will lie within Dr. Kreipke's team of experts which includes Dr. Dore-Duffy (an expert in hemodynamics), Dr. Kuhn (an expert in pharmacology), Dr. Rafols (an expert in the TBI model) and Dr. Meuller (an expert in cardiovascular control). However, Dr. Armstead will be included in Year 1 at 15% to allow for him to be involved in all data interpretation as the data gleaned from Year 1 will, in part, impact the direction of subsequent years. Dr. Armstead will have full access to data and we will meet on a biweekly basis by phone/video conference and twice throughout the year in person.

The second and third years of this application will be conducted in pig only. Therefore, most of the responsibilities will lie within Dr. Armstead's team of experts which includes Joel Greenberg (an expert in hemodynamics) and Susan Margulies (an expert in pig behavior). In these years Dr. Kreipke will be retained at 15% to allow for involvement in interpreting and writing up the obtained results. He, too, will meet on a biweekly basis to discuss data and will meet twice in person to aide in data transfer and continuity.

Drs. Kreipke and Armstead have been in discussion for over one year regarding specifically this proposal. In this time, both have solidified their own respective teams. Further, negotiations with Actelion regarding material transfer and intellectual rights were accomplished in this year (copy of MTA included in appendix). While Dr. Kreipke is directly implicated in the MTA, he will allow free and clear access to Clazosentan as needed specifically for this proposal. Drs. Kreipke and Armstead have also begun working together on projects related to endothelin, the results of which will be published in an up-in-coming special edition of Neurological Research.

Conflict Resolution

While Drs. Kreipke and Armstead have an established working relationship and are both highly motivated to see the completion of all work and successful application to IND, both investigators have preemptively anticipated steps to resolve any conflict in interpretation of data that may arise. Drs. Harry Goshgarian from Wayne State University (an expert in spinal cord injury) and Douglas Smith from University of Pennsylvania (an expert in pig traumatic brain injury) will be available as needed as an independent set of investigators to aide in resolving any conflicts that may arise. If conflict arises, both multi-PIs will meet weekly with Drs. Goshgarian and Smith to resolve and issues in a timely and scientifically-driven manner. Experience shows, however, that the joint collaboration between Drs. Kreipke and Armstead is likely to be highly successful.

Form Approved Through 6/30/2012 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Clazosentan: A novel treatment of traumatic brain injury

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PAR-08-233 Title: NINDS Cooperative Program in Translational Research Single-Component Research Projects (U01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Armstead, William M.	PhD	ARMSTEADW

3c. POSITION TITLE
Research Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Dept. of Anesthesiology and Critical Care
3620 Hamilton Walk; 339 John Morgan Bldg
Philadelphia, PA 19104-6112.

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Anesthesia

3f. MAJOR SUBDIVISION
School of Medicine

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 215-573-3674 FAX: 215-349-5078

E-MAIL ADDRESS:
william.armstead@uphs.upenn.edu

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☐ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
	☐ No ☐ Yes	☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes — 5a. Animal Welfare Assurance No. A3079-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
12/01/10	11/30/14	$28,263	$45,222	$1,555,508	$2,443,812

9. APPLICANT ORGANIZATION
Name: Trustees of the University of Pennsylvania
Address: Office of Research Services
3451 Walnut Street, Rm. P-221
Philadelphia, PA 19104-6205

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1231352685A1
DUNS NO. 04-225-0712 Cong. District PA-002

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Pamela Caudill
Title: Executive Director
Address: Office of Research Services
3451 Walnut Street, Rm. P-221
Philadelphia, PA 19104-6205
Tel: 215-898-9984 - FAX: 215-898-9708
E-Mail: pennaors@lists.upenn.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Marianne Achenbach
Title: Director, Research Services
Address: Office of Research Services
3451 Walnut Street, Rm. P-221
Philadelphia, PA 19104-6205
Tel: 215-573-8798 FAX: 215-898-9708
E-Mail: pennaors@lists.upenn.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* DATE: 1/26/10

WAYNE STATE UNIVERSITY
SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMY
AND CELL BIOLOGY

January 25, 2010

To the Review Committee:

This letter is written to enthusiastically support the efforts of Dr. Kreipke in his pursuit of the U01 proposal, "Clazosentan: a novel treatment for traumatic brain injury". While Dr. Kreipke is a junior faculty member in the Department of Anatomy he has proven to be a highly productive individual with a keen sense of organizational and time management skills. He also has proven to possess extraordinary independence and leadership skills for such a young individual. Proof positive of this includes the facts that he has established and continues multiple collaborations across departmental boundaries and manages a laboratory of his own with three research assistants, three rotating medical students and two undergraduate researchers. This, combined with his own vitality, should allay any concerns as to whether Dr. Kreipke is suited to carry out the proposed work together with his already funded projects.

As Chair of the Department, I am committed to support the continued success of Dr. Kreipke and will work, in conjunction with both the School of Medicine and the Office of the Vice President for Research to provide additional support as necessary to facilitate this exciting project.

Sincerely,

Linda D. Hazlett, Ph.D.
Distinguished Professor and Chair

LDH/slh

540 EAST CANFIELD AVENUE ◊ GORDON H. SCOTT HALL OF BASIC MEDICAL SCIENCES ◊ DETROIT, MI 48202 ◊ (313) 577-1061 ◊ FAX (313) 577-3125

Daniel A. Walz, Ph.D.
Office of Research
School of Medicine
Scott Hall, Suite 1261
540 East Canfield
Detroit, MI 48201
Tel. (313) 577-9553

January 25, 2010

To the Review Panel:

RE: U01 Proposal entitled, "Clazosentan: A Novel Treatment of Traumatic Brain Injury"
Contact PI – Christian W. Kreipke, Ph.D., Wayne State University
Multiple PI – William Armstead, Ph.D., University of Pennsylvania

We are very excited about the prospect of this type of research being conducted at Wayne State University in conjunction with University of Pennsylvania. Dr. Kreipke is a very bright and energetic young individual who, in my assessment, is more than capable of carrying out this work, especially in light of the extensive collaborations he has managed to secure throughout multiple departments in the School of Medicine.

The Office of Research at Wayne State University's School of Medicine is committed to assisting Dr. Kreipke as needed in his pursuit of this award. Upon successful funding we will continue this support both in time and resources. Furthermore, upon granting of IND status, my office is committed to working in close association with our Clinical Departments to assist in the transition into implementation of Clazosentan into human trial. We have already identified key faculty and staff who have extensive experience in clinical trials who will assist Dr. Kreipke. We wish him all the very best in the review process.

Sincerely,

Daniel A. Walz, Ph.D.
Associate Dean for Research
Wayne State University, School of Medicine

ACTELION

Dr. Christian Kreipke

Assistant Professor, Anatomy

and Cell Biology

Wayne State University, School

of Medicine, Detroit, USA

Allschwil, 20 January 2010

Dear Dr. Kreipke,

We are very interested in your proposal to test the efficacy of clazosentan in animals models of traumatic brain injury (TBI) and will continue to provide you with clazosentan under a material transfer agreement. Such a study is the logical continuation of your promising early findings obtained with peptidic endothelin receptor antagonists in the same models. Positive outcome with clazosentan in your models would encourage us to envisage such an indication. Furthermore, the existing preclinical package including DMPK, safety pharmacology and toxicology of clazosentan would be appropriate since this new indication is close to SAH for which clazosentan is already in phase III.

Yours sincerely,

Marc Iglarz

Pharmacology and Preclinical development

Actelion Pharmaceuticals LTD.

Actelion Pharmaceuticals Ltd
Gewerbestrasse 16 | CH-4123 Allschwil | Switzerland | phone +41 61 565 65 65 | fax +41 61 565 89 03 | marc.iglarz@actelion.com
|www.actelion.com
Address for visitors: Hegenheimermattweg 91

WAYNE STATE UNIVERSITY

Harry Goshgarian, PhD
Professor
Anatomy and Cell Biology
9304 Scott Hall
540 East Canfield
Detroit, MI 48201
hgoshgarian@med.wayne.edu
(o) 313.577.1045
(f) 313.577.3125

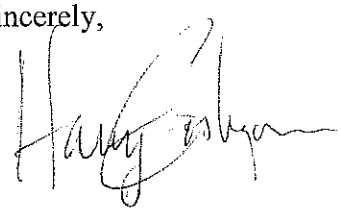
January 20, 2010

Christian Kreipke, PhD
Assistant Professor
Anatomy and Cell Biology

Dear Christian:

I have thoroughly enjoyed reading your application, "Clazosentan: A novel treatment of traumatic brain injury". I, therefore, would be happy to serve on your "conflict resolution" committee should any disparity occur between you and Dr. Armstead over the interpretation of your results. Furthermore, I would be happy to assist you in any capacity during the tenure of this project. If you have any concerns, please feel free to contact me.

Sincerely,



Harry Goshgarian, PhD

Penn
University of Pennsylvania

University of Pennsylvania School of Medicine
Hospital of the University of Pennsylvania
Department of Neurosurgery

Penn Center for Brain Injury & Repair
Douglas H. Smith, M.D.
Director, Penn Center for Brain Injury & Repair
Robert A. Groff Professor of Neurosurgery
Vice Chairman for Research & Education, Department of Neurosurgery

January 27, 2010

William M. Armstead, Ph.D.
University of Pennsylvania
Department of Anesthesiology and Critical Care
3620 Hamilton Walk, JM3
Philadelphia, PA 19104-4283

Re: Clazosentan: A Novel Treatment Of Traumatic Brain Injury

Bill,

I have read the above grant proposal, and I am enthusiastic regarding your Aims and Goal in testing whether clazosentan is effective in ameliorating hypoperfusion and improving behavioral outcome following traumatic brain injury. It will be my pleasure to serve as a Consultant on the Advisory Board for this Multi-PI UO1 proposal.

Warm wishes,

Douglas H. Smith, M.D.
smithdou@mail.med.upenn.edu